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THE ROLE OF THE LIVER AND KIDNEYS IN THE METABOLISM OF INTRAVENOUS TESTOSTERONE BY HUMAN SUBJECTS*†

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INTRODUCTION

THE authors have previously reported the metabolism of intravenous testosterone by experimental animals (1). The present report is concerned with the metabolism of intravenous testosterone in human subjects.

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EXPERIMENTAL

Solutions of testosterone in 25 per cent human serum albumin[1] were prepared as previously described (1). To reduce bacterial contamination the solutions were prepared twelve hours prior to use and sterile techniques were employed throughout. Bacteriostatic amounts of penicillin and streptomycin were added to the solutions. The maximum concentration of testosterone in 25 per cent human serum albumin obtained in this way was 2.31 mg./ml.

These testosterone-albumin solutions were administered to a total of 28 human subjects, both normal and diseased. Prior to administration each subject received a complete physical examination with special attention paid to the cardiovascular system in order to avoid cardiac embarrassment from the rapid administration of 100 ml. of 25 per cent human serum albumin. Pyrogenic reactions which occurred in 2 instances lasted a maximum of one hour with no complications. There were no unfavorable reactions of any nature in the other 26 subjects.

The solutions were given over 10-minute to 20-minute periods in doses ranging from 147 to 186 mg. of testosterone. The mid-point of administration was taken as zero time in the experiments. Blood samples were drawn periodically thereafter. Urine was collected during specified time intervals.

Previous investigators have shown that liver and kidney tissues are active in the catabolism of testosterone (2, 3, 4). In order to observe the effect of the liver, a comparison was made between the metabolism of testosterone in normal subjects and in patients with liver disease. Similarly, subjects with kidney disease were used to study the effect of the kidneys. To test the effect of wasting diseases on the metabolism of intravenous testosterone patients in the terminal phase of malignancy were studied. A male patient who had been castrated five years previously was also given testosterone intravenously to determine the effect of an absence of testes on its metabolism.

Free steroids in the blood

Known volumes (approx. 20 ml.) of oxalated blood were hemolyzed by the addition of equal volumes of distilled water. The samples were then extracted with ether. The ether extracts were purified for analysis by chromatography on alumina and partitioning between hexane and 70 per cent ethanol, as described in a previous publication (5). Quantitative analyses were made on these purified extracts for α, β unsaturated ketosteroids by means of the absorption band at 240 mμ in the ultraviolet, and

[1] The testosterone was furnished by Ciba Pharmaceutical Products, Inc., and the serum albumin used in this study was processed by the American Red Cross from blood which it collected from volunteer donors. The solutions of testosterone in serum albumin were prepared by Armour and Company, through the courtesy of Dr. Edwin E. Hays.

for 17-ketosteroids by the Callow modification of the Zimmermann reaction (6).

"Conjugated" steroids in the blood

The blood samples after ether extraction were submitted to hydrolysis in order to release any conjugated steroids present in the blood.

Four volumes of 95 per cent ethanol were added to one volume of hemolyzed blood. The precipitated proteins were removed by centrifugation and the precipitate washed four times with 10-ml. portions of 95 per cent ethanol. The ethanol washes were combined with the protein-free supernatant liquid and the alcohol removed *in vacuo*. The protein precipitate was discarded.

The aqueous "protein-free" solution was diluted to 100 ml. with distilled water and 10 ml. of concentrated HCl were added. The mixture was boiled under reflux for fifteen minutes, after which it was quickly cooled and extracted five times with equal volumes of ether. The ether extracts were washed with 2.5 N sodium hydroxide, 8 N sulfuric acid, and water, and evaporated to dryness. These extracts were then subjected to further purification by chromatography and partitioning between solvents as described by Samuels (6). The final products were analyzed quantitatively for α, β unsaturated ketones and 17-ketosteroids in the usual manner.

This method of hydrolysis of blood differs from the one used in a previous study (1) in which the recovery of androsterone sulfate added to blood was only 51 per cent. A comparison was made between the two methods as follows: A male subject was injected intravenously with 180 mg. of testosterone in albumin. Forty-five minutes after injection a single blood specimen of 200 ml. was taken. Three 20-ml. samples were analyzed for 17-ketosteroids by the old method and three samples by the new method. The old method yielded $278 \pm 12\gamma$ per 100 ml. of 17-ketosteroids, whereas the new method yielded an average of $514 \pm 9.5\gamma$ per 100 ml. Thus it would appear that this method of hydrolysis is to be preferred to the previous method.

Urine analyses

Urine samples collected during specified intervals of time after testosterone administration were systematically examined for their α, β unsaturated ketosteroid and their 17-ketosteroid content as described in a previous report (1).

RESULTS

As in experimental animals, testosterone administered intravenously to human subjects disappeared very rapidly from the circulation. In Figure 1 the percentage of the "original blood concentration" of testosterone in the

blood of subject CE is plotted against time after injection. The "original blood concentration" is that theoretical concentration of testosterone that would be found if none of the testosterone were lost from the circulation or destroyed. It is calculated from the estimated blood volume and the amount of testosterone given. Only 7.5 per cent of the expected concentration could

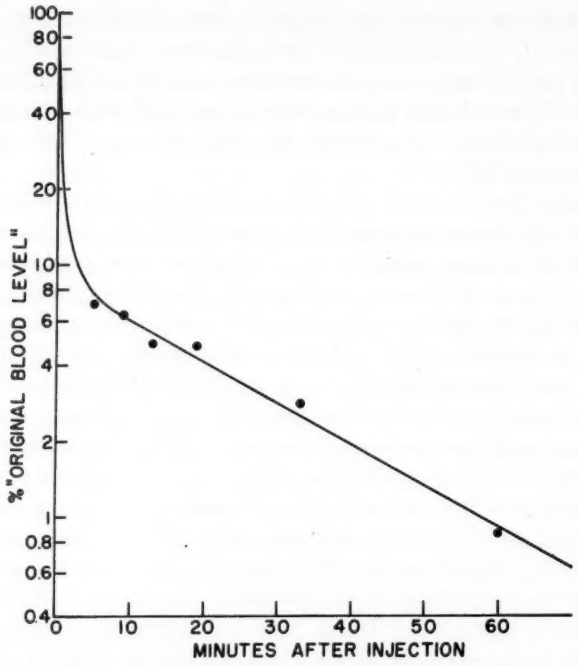

FIG. 1. Levels of testosterone in the blood of subject CE after the intravenous injection of 192 mg. of hormone in 25 per cent serum albumin solution.

be demonstrated five minutes after administration. The decline of testosterone concentration in the blood followed a logarithmic course after the first rapid loss of testosterone. By sixty minutes after the injection the blood concentration had fallen to a level which was just within the range of analysis (Table 1).

Although the series of cases in this study is not large, the results indicate that the various diseased states had little influence on the rate of disappearance of circulating testosterone. In each case less than 10 per cent of the expected blood level was found ten minutes after administration.

No measurable amounts of 17-ketosteroids could be detected in the extracts of unhydrolyzed blood samples taken after the administration of

testosterone. When these samples were hydrolyzed, however, 17-ketosteroids were demonstrable. Samples of blood from normal subjects before the injection of the hormone had no demonstrable 17-ketosteroids by this method, either before or after hydrolysis.

In order to determine which portion of the whole blood carried the 17-ketosteroids, a 400-ml. sample of blood was collected in oxalate from a normal subject fifty minutes after the testosterone administration. From this single sample, 17-ketosteroid analyses were run in triplicate on both

TABLE 1. LEVELS OF TESTOSTERONE IN BLOOD FOLLOWING INTRAVENOUS ADMINISTRATION IN NORMAL AND DISEASED HUMAN SUBJECTS

Subject	Diagnosis	Testosterone, γ per 100 ml. of blood per unit dose*			
		10 min.	20 min.	30 min.	60 min.
CE	Normal	158	107	71	21
CN	Normal	153	128	102	28
CR	Normal	—	199	31	10
CG	Cirrhosis	187	112	57	21
CK	Cirrhosis	158	86	53	51
CL	Cirrhosis	182	152	123	34
CW	Nephritis	—	—	159	76
CZ	Nephritis	—	—	57	23
CC	Carcinomatosis	215	135	77	10
DA	Carcinomatosis	—	—	116	41
CX	Male castrate	—	—	84	16

* Unit dose = 1 mg. of testosterone per pound of body weight.

plasma and whole blood. When corrections for the hematocrit were made on the values for whole blood, it was found that at least 96 per cent of the 17-ketosteroids were in the plasma and no more than 4 per cent were associated with the red cells and other formed elements of the blood. It is highly probable that all of the conjugated steroid is in the plasma.

Table 2 lists the levels of conjugated 17-ketosteroids found in plasma at various intervals after the injection of testosterone. Seven normal patients, 4 patients with liver disease, and 1 patient with kidney disease were included. From these results it is apparent that the administered testosterone was very rapidly metabolized to conjugated 17-ketosteroids in the normal subjects. In the majority of the cases the earliest specimens, twenty minutes after injection, had the highest 17-ketosteroid concentrations. When these plasma concentrations are plotted against time (Fig. 2), it would appear from the shape of the curves that the maximum concentra-

tion of 17-ketosteroids in the blood occurred even earlier than twenty minutes after injection. The rate of fall thereafter was proportionate to the concentration.

The total amount of 17-ketosteroids in the circulation can be roughly approximated from their concentration in plasma and the total plasma volume, assuming that this is equal to 5 per cent of the body weight. The average total amount of 17-ketosteroids in the plasma of 4 normal subjects

TABLE 2. LEVELS OF CONJUGATED 17-KETOSTEROIDS IN PLASMA FOLLOWING INTRAVENOUS TESTOSTERONE IN NORMAL AND DISEASED HUMAN SUBJECTS

Subject	Diagnosis	17-Ketosteroids,* γ per 100 ml. of plasma per unit dose**					
		20 min.	40 min.	60 min.	1½ hrs.	2 hrs.	3 hrs.
DL	Normal	726	630	540	437	360	238
DQ	Normal	348	403	349	329	300	225
DS	Normal	576	470	382	277	240	170
DT	Normal	525	390	278	177	155	107
	Average	*544*	*475*	*387*	*305*	*264*	*185*
CR	Normal		445				
CH	Normal		475				
DK	Normal			463			
DM	Hepatitis	180	122	108	166	160	182
DO	Cirrhosis	181	218	251	302		
DR	Cirrhosis	141	146	163	158	149	
	Average	*167*	*162*	*174*	*209*		
CK	Cirrhosis			24			
CZ	Nephritis			812			

* As androsterone.

** Unit dose = 1 mg. of testosterone per pound of body weight.

receiving an average dose of 167 mg. of testosterone was 18.9 mg., twenty minutes after the injections. This represented 11 per cent of the injected testosterone. It was probable that more had been metabolized to 17-ketosteroids and either excreted into the urine or stored in tissues.

The concentrations of 17-ketosteroids in plasma after testosterone was injected intravenously into normal subjects were much higher than in patients with liver disease (Table 2). At twenty minutes after injection

the average concentration in 4 normal subjects was 544 micrograms per 100 ml. of plasma; whereas, in 3 cases of liver disease the concentration was 167 micrograms per 100 ml. The concentration in the blood of these patients did not decrease logarithmically with time, as in the normals, but actually increased during the first ninety minutes (Fig. 2). Various clinical

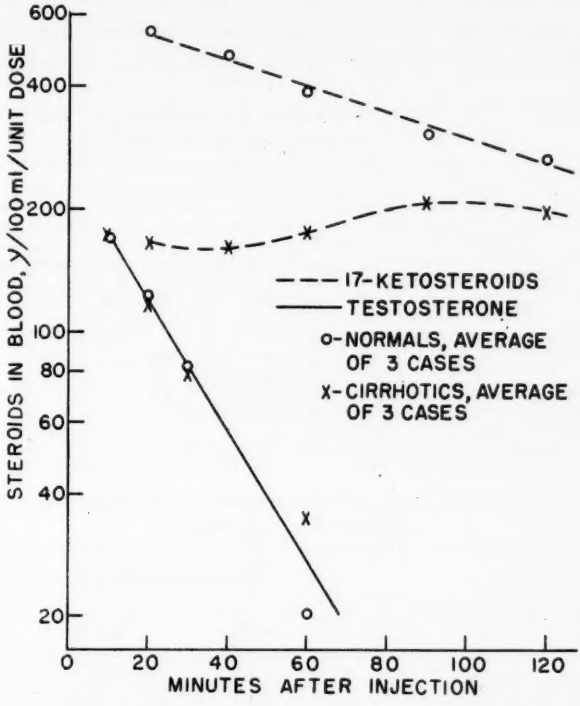

FIG. 2. Average levels of testosterone and total 17-ketosteroids in the blood following intravenous injection of testosterone into normal subjects and patients with liver damage.

tests showed that all these patients had impaired liver function. Although only a few patients were used, this marked difference indicates that the ability to metabolize testosterone to 17-ketosteroids had been altered in the patients with liver disease.

The highest concentration of 17-ketosteroids in plasma was obtained in a case of severe glomerulonephritis. At one hour after injection the plasma level was 812 micrograms per 100 ml. of plasma, as compared to an average value of 402 micrograms per 100 ml. in the normal subjects. An endogenous creatinine clearance run simultaneously with this experi-

ment showed that the amount of plasma which was cleared per minute was only 13.5 ml. Apparently the elevated plasma 17-ketosteroid level was principally the result of this reduction in glomerular filtration.

Urinary excretion of α, β unsaturated ketonic steroids after intravenous testosterone

In these experiments the urines also were examined for their α, β unsaturated ketosteroid content. Unhydrolyzed urines were first extracted

TABLE 3. FREE AND CONJUGATED α, β UNSATURATED STEROIDS IN THE URINE AFTER INTRAVENOUS TESTOSTERONE

Subject	Diagnosis	Mg. testosterone I.V.	Urinary α, β unsaturated steroids, as γ of testosterone										
			1st hr.		2nd hr.		3rd hr.		4th hr.		5th hr.		Total
			free	conj.	free	conj.	free	conj.	free	conj.	free	conj.	in mg.
CE	Normal	162	107	1100	40	513	10	202	0	91	0		2.06
CN	Normal	147	20	1051	4	577	0						1.65
CR	Normal	168	185	1306	20	566	0						2.08
CV	Normal	165	23	602	3	478	0	373		206			1.68
CH	Normal	188				515		14		0	0		
CW	Nephritis	169	49	552	0	595	0	321					1.52
CZ	Nephritis	164	0	0	0	0							0
CX	Male castrate	186	101	1317	0								1.42

TABLE 4. FREE 17-KETOSTEROIDS IN URINE AFTER INTRAVENOUS TESTOSTERONE

Subject	Diagnosis	Free 17-ketosteroids excreted (γ)		
		1st hr.	2nd hr.	3rd hr.
CN	Normal	172	35	0
CR	Normal	185	47	0
CV	Normal	24	5	0
CW	Nephritis	0	0	0
CZ	Nephritis	42	21	0
CX	Male castrate	119	—	—

with ether and then hydrolyzed in the usual manner and again extracted with ether. In this manner the "free" steroids were separated from the "conjugated" steroids.

Table 3 shows the α, β unsaturated ketosteroid content of urines after intravenous administration of testosterone to 5 normal subjects, 2 nephritic patients, and 1 male castrate. These results indicate that after intravenous testosterone a substance having maximum absorption at 240 mμ, which was not present prior to administration, appeared in the urine. Presumably

this was testosterone, but it may have been any other α, β unsaturated ketosteroid resulting from testosterone metabolism. Considering the dose of testosterone administered, only a small amount (less than 2 per cent) was excreted into the urine.

TABLE 5. DAILY URINARY EXCRETION OF 17-KETOSTEROIDS BEFORE AND AFTER INTRAVENOUS TESTOSTERONE

Normal subject CE		Normal subject CR		Carcinomatous subject CC	
Exper. day	17-KS, mg./24 hrs.	Exper. day	17-KS, mg./24 hrs.	Exper. day	17-KS, mg./24 hrs.
1	11.2	1	7.3	1	17.6
2	13.6	2	8.8	2	25.7
3	11.2	3	9.9	3	12.1
4	12.3	4	7.2	4	19.3
5	11.0			5	18.4
6	11.6			6	15.6
Control average	*11.8*	*Control average*	*8.3*	*Control average*	*18.1*
7*	128.0	5**	109.4	7†	106.0
8	14.6	6	11.8	8	31.5
9	11.7	7	11.5	9	19.8
10	11.7	8	9.9	10	18.3
11	14.3	9	14.8	11	29.3
12	13.6	10	16.9	12	24.4
13	12.8	11	12.3	13	13.4
14	8.2	12	15.2	14	16.4
15	10.4				
16	11.5				
Post-injection average	*12.1*	*Post-injection average*	*13.2*	*Post-injection average*	*23.3*

* Day of injection: 162 mg. of testosterone i.v.
** Day of injection: 188 mg. of testosterone i.v.
† Day of injection: 164 mg. of testosterone i.v.

"Free" 17-ketosteroids in the urine after intravenous testosterone

Small but definite amounts of free 17-ketosteroids were demonstrable in the ether extracts of unhydrolyzed urines following intravenous testosterone (Table 4). These were not present in measurable amounts before injection of the hormone.

Conjugated 17-ketosteroids in the urine after intravenous testosterone

Large amounts of conjugated 17-ketosteroids were demonstrated in the ether extracts of hydrolyzed urines of subjects receiving intravenous testosterone. The urinary 17-ketosteroid excretion before and after testosterone administration was studied in 3 subjects (Table 5). In normal subject CE 71.5 per cent of the administered testosterone was excreted as excess conjugated 17-ketosteroids in the urine during the first twenty-four hours after injection. Subsequently another 2.6 per cent was excreted, accounting for a total of 74.1 per cent of the testosterone. In normal subject CR these figures were 53.9 per cent in the first twenty-four hours and 18.3 per cent in the subsequent period, making a total of 72.2 per cent. In the carcinomatous patient CC, 53.5 per cent was excreted in the first twenty-four hours and 22.2 per cent subsequently, making a total of 75.7 per cent. In these 3 subjects, an average of 74 per cent of the injected testosterone was accounted for by an increase in urinary 17-ketosteroids. An average of 63.1 per cent was excreted during the first twenty-four hours after injection.

In every one of these 3 subjects the excretion of 17-ketosteroids returned to the normal range by the third day after injection and then showed a secondary rise on the fifth day which continued for two to three days. This secondary rise is probably associated with the metabolically active steroid, since nitrogen storage continues for eight to ten days after the single injection (7).

Hourly urinary 17-ketosteroids after intravenous testosterone

After the administration of testosterone urine was collected hourly to determine when the peak excretion of 17-ketosteroids occurred. From the data in Table 6 it is evident that the peak excretion occurred during the first and second hours after injection in the normal subjects.

Effect of liver disease on the rate of excretion of 17-ketosteroids after intravenous testosterone

Data on the 17-ketosteroid excretion in cirrhotic subject CG (Table 6) indicated that the rate of metabolism of testosterone to a 17-ketosteroid might be decreased in liver disease, as reflected by a lower than normal excretion in the first and second hours after injection. To test this assumption, a series of 10 normal subjects and 12 patients with liver disease were given testosterone intravenously. Urines collected during the first and second hours after injection were analyzed for total 17-ketosteroids. One case of chronic glomerulonephritis was similarly examined.

Inasmuch as patients with severe liver disease frequently have a reduced

renal clearance, simultaneous endogenous creatinine clearances were run on most of these subjects to estimate the influence of the kidney on the urinary excretion of the 17-ketosteroids (Table 7). Normal human subjects were able to convert intravenous testosterone to 17-ketosteroids at a much more rapid rate than patients with liver disease. In 10 normal sub-

TABLE 6. HOURLY URINARY EXCRETION OF TOTAL 17-KETOSTEROIDS AFTER INTRAVENOUS TESTOSTERONE

Hrs. after injection	Normal subject CE		Normal subject CH		Cirrhotic subject CG	
	17-KS, mg./hr.	% Testosterone excreted as 17-KS	17-KS, mg./hr.	% Testosterone excreted as 17-KS	17-KS, mg./hr.	% Testosterone excreted as 17-KS
1	29.6	17.7	35.1	18.4	6.4	3.6
2	31.3	19.0	22.3	11.6	13.1	8.4
3	15.9	9.5	12.3	6.3	11.5	7.3
4	10.4	6.1	11.3	5.8	5.7	3.1
5	10.5	6.2	5.3	2.5	4.8	2.5
6	6.4	3.6	3.6	1.6	1.8	0.4
7			3.4	1.5	3.8	1.8
8			2.9	1.2	2.2	0.7
9			3.1	1.3		
10			1.2	0.4		

jects an average of 16.4 per cent of the administered testosterone was excreted in this form during the first hour after injection, as compared to an average of 4.6 per cent for 12 patients with liver disease. During the second hour the excretion was 14.6 per cent in the normal subjects and 5.6 per cent in cirrhotic patients.

The endogenous creatinine clearance averaged 123 ml. of plasma cleared per minute in 7 normal subjects, and 99 ml. per minute in 7 patients with liver disease. This was an average reduction of 19.5 per cent in creatinine clearance in liver disease, against an average reduction of 72 per cent in the excretion of 17-ketosteroids. Evidently reduced glomerular filtration does not entirely account for the reduction in 17-ketosteroid excretion in liver disease after intravenous administration of testosterone.

In order to determine which kidney mechanism was involved in the excretion of conjugated 17-ketosteroids, the plasma clearances were calculated in 4 normal subjects and in 3 patients with liver disease in whom plasma 17-ketosteroid values were available. The average two-hour urinary

TABLE 7. A COMPARISON OF THE RATE OF EXCRETION OF 17-KETOSTEROIDS BETWEEN NORMAL SUBJECTS AND PATIENTS WITH LIVER DISEASE

Subject	Diagnosis	% Administered testosterone excreted as 17-KS		Creatinine clearance in ml. of plasma per. min.
		1st hour	2nd hour	
CE	Normal	17.7	19.0	123
CH	Normal	18.4	11.6	152
CN	Normal	15.4	16.5	108
CR	Normal	18.1	17.7	111
CV	Normal	12.2	10.1	117
DB	Normal	11.7	16.7	123
DC	Normal	13.2	10.5	130
DG	Normal	13.5	17.5	
DL	Normal	18.0	12.9	
DS	Normal	25.7	13.8	
	Normal averages	*16.4*	*14.6*	*123*
CG	Cirrhosis	4.1	8.9	
CL	Cirrhosis	8.1	10.4	165
CM	Cirrhosis	0.2	3.5	65
CP	Cirrhosis	6.1	6.9	115
CQ	Cirrhosis	2.6	2.7	69
CS	Cirrhosis	6.9	—	92
CT	Cirrhosis	1.9	3.2	77
CU	Cirrhosis	5.2	5.5	66
DM	Hepatitis	11.3	8.1	
DO	Cirrhosis	4.4	10.8	
DR	Cirrhosis	4.3	6.8	
CW	?Ca. of liver & nephritis	0.7	0.9	141
	Liver disease averages	*4.6*	*5.6*	*99*
CZ	Chronic glomerulonephritis	1.4	1.5	14

excretion values and the average plasma 17-ketosteroid values at sixty minutes were used for the calculations. Using the average plasma concentration value at sixty minutes was valid, inasmuch as a plot of the logarithm of the concentrations at serial times in the normal subjects followed a straight line during this two-hour period of excretion. This was not quite true in the case of the cirrhotic patients, but the variation was not sufficient to invalidate the calculations since the levels were almost constant.

In this manner the renal plasma clearance of 17-ketosteroids in the 4 normal subjects was calculated to be 126 ml. per minute. In the 3 patients with liver disease this figure was 100 ml. per minute. There is close agreement between the creatinine clearances and the 17-ketosteroid clearances in both groups. Although the number of cases studied is small, it seems probable that the conjugated 17-ketosteroids are excreted by glomerular filtration without significant reabsorption or secretion.

DISCUSSION

One of the most striking findings in this investigation was the rapid rate of disappearance of the intravenous testosterone. Within five minutes after injection less than 10 per cent of the theoretical blood level of testosterone was found. Referring to the animal experiments (1), this early loss of testosterone from the circulation could be explained by diffusion of the testosterone into the tissues.

Once equilibrium was established, the rate of fall of the testosterone concentration in the blood was logarithmic, a result one would expect if this concentration were the limiting factor in the rate of the enzymic reactions. The work of Sweat *et al.* (8) has shown that there are two major enzymes in the liver which act on the testosterone molecule: one requiring citrate as a cofactor which acts on the conjugated system in ring A without affecting the alcohol group on C-17, and another requiring DPN for its action which first oxidizes the alcohol to a 17-ketone and then, by further enzymic reactions, reduces ring A to a saturated alcohol. From *in vitro* experiments it was concluded that the latter route, producing androsterone and its isomers, was the preferred route in the human body.

Since, in the normal individual, the concentrations of both testosterone and 17-ketosteroids fall at a logarithmic rate over the measurable range, it would appear that none of the intervening enzyme systems is saturated. Since the concentration of 17-ketosteroids is the algebraic result of their formation and their removal by the kidney, it must be true that the latter process is also proportionate to the concentration, a condition borne out by the renal clearances which indicate that these substances are filtered without being reabsorbed. Assuming a constant blood flow, the result observed would be anticipated. Further, one can conclude that the reaction involving conjugation must have its equilibrium far on the side of the conjugated product, since the conversion of testosterone to these compounds was still only dependent on the testosterone concentration when the concentration of the conjugates was almost 20 times that of the hormone (Fig. 2).

The rate of disappearance of testosterone cannot, however, be taken as a measure of the rate of the limiting reaction in the formation of 17-

ketosteroids, because the data also show that the hormone is disappearing by some other route. This is probably the citrate system which was found in the *in vitro* studies with liver minces. It seems to occupy a minor role in the normal human being, however, under these circumstances since most of the injected hormone could be accounted for in the conjugated 17-ketosteroids.

The role of the liver in these enzymic mechanisms is emphasized by the results in the cirrhotic patients. During the first sixty minutes the concentration of testosterone decreased along the same logarithmic line followed in the normal subjects. The concentration of 17-ketosteroids in the blood, however, followed an altogether different course from that in the normal individual. Instead of a logarithmic decrease from a high level, it was about one-fourth the normal level twenty minutes after injection and rose at an increasing rate thereafter for the first hour. The slow production was reflected in a very low excretion during the first hour, increasing during the second hour, and approximating the decreasing normal rate from the third hour on.

This type of curve could only be obtained with a falling testosterone level if some intermediate were accumulating, the breakdown of which is normally so rapid that it is not a limiting factor in the over-all reaction. As the concentration of this factor rises, the conversion to 17-ketosteroids increases by mass action until the rate equals that of the normal without accumulation of the intermediate. Thus damage to the liver markedly decreases the activity of one or more of the enzymes involved in the conversion of testosterone to 17-ketosteroids. The effect appears to be on a step beyond the first change in the testosterone molecule.

The limiting reaction in the cirrhotic patients did not appear to be the conjugation reaction. If the decreased 17-ketosteroid excretion in patients with liver disease were caused by a decrease in the rate of conjugation, it would be expected that free 17-ketosteroids would accumulate in the tissues and blood, assuming that the 17-ketosteroids were not subjected to further metabolic breakdown. No evidence of increased free 17-ketosteroids in the urine or plasma of cirrhotic patients was found. It is possible that the 17-ketosteroids accumulated in the tissues and were not detected but this seems highly unlikely, since then a higher than normal excretion would have been expected as the 17-ketosteroids returned to the circulation.

This decrease in the usually preferred path of testosterone metabolism apparently results in an increased disappearance by other routes, for much less of the injected hormone could be accounted for by increased excretion of 17-ketosteroids. The total excess 17-ketosteroid excretion in patients with liver disease is only approximately one third of that in

normals, with a large degree of variation depending upon the severity of the abnormality. This indicates that in liver disease a large part of the administered testosterone is metabolized to other metabolites than 17-ketosteroids. These may be steroidal or nonsteroidal. Unfortunately this investigation made no attempt to establish the urinary steroid pattern in the patients with cirrhosis by identification of the excreted steroids, nor were the feces analyzed.

The kidney is known to contain enzymes capable of metabolizing testosterone (4). Yet in kidney diseases, 17-ketosteroid formation still occurred at a rapid rate after the intravenous injection of testosterone, as evidenced by the very high 17-ketosteroid levels in plasma. Obviously the exact role of the kidney in relation to the liver in the over-all metabolism of testosterone by the whole body cannot be determined from these data. Previous experiments with hepatectomized, nephrectomized animals injected intravenously with testosterone would indicate that other tissues besides liver and kidney must be capable of metabolizing testosterone (1). No way of testing this hypothesis in human subjects was found in these experiments.

SUMMARY

Testosterone dissolved in human serum albumin was given intravenously to human subjects and its metabolism studied. In normal subjects an average of 74 per cent of the injected testosterone was accounted for by an increase in urinary conjugated 17-ketosteroids during the post-injection period. An average of 63 per cent was excreted during the first twenty-four hours, with the major part of this excretion occurring during the first two hours. Following intravenous testosterone, conjugated 17-ketosteroids were demonstrable in the plasma. Maximum plasma levels were found as early as twenty minutes after administration.

The importance of the liver in the metabolism of testosterone was demonstrated by the lower concentrations of 17-ketosteroids in both the urine and plasma following administration of testosterone intravenously in a series of patients with liver disease. More than one pathway of testosterone metabolism was indicated by the much lower proportion of the injected hormone which was recovered as excess 17-ketosteroids in the cirrhotic patients in spite of the rapid disappearance and minimal excretion of the original steroid.

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THE METABOLIC EFFECTS OF ADRENOCORTICOTROPIC HORMONE (ACTH) IN A PATIENT WITH CUSHING'S SYNDROME AND ACROMEGALY*†

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INCREASED function of the adrenal cortex in acromegaly has been suggested by the occurrence in this condition of osteoporosis, diabetes, enlarged adrenals and, occasionally, of hypertension, hirsutism and increased adrenal steroid excretion. The simultaneous development of classic Cushing's syndrome and acromegaly in a patient has offered an unusual opportunity to investigate the relationship of these two endocrine abnormalities. The metabolic studies which were carried out on this patient form the subject of this paper.[1] A detailed clinical description of the case has been published elsewhere (1).

METHODS

The patient was given a constant, low-calcium diet. Duplicate diets were analyzed at intervals throughout the study and the results averaged. Fifty per cent aliquots of each duplicate diet were homogenized and made up to volume. The butter (salt-free) was included to stabilize the homogenate, the volume of solids thus contributed being negligible. Feces were collected in six-day pools, using carmine to mark the last meal of each period. The pools were homogenized and their volumes measured. Aliquots of the diet and stool homogenates were dry-ashed at 440° C. for sodium, potassium and calcium analyses. Aliquots of the homogenates were also taken for nitrogen, phosphorus and chloride analyses, respectively. All analyses were performed in duplicate.

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[1] We are indebted to Drs. R. V. McCormick, C. E. Reed and R. H. Murray, and to the Roosevelt Hospital, New York, for referring the patient to us.

Sodium and potassium determinations on dry ashes, urines, and serum were made with a flame photometer, using lithium as an internal standard (2). Dry-ash and urinary calciums were determined by the method of Fiske and Logan (3), and serum calcium by the method of Clark and Collip (4).[2]

Nitrogen was determined by a modified Kjeldahl technique. Total phosphorus in the homogenates and inorganic phosphorus in serum[2] and urine[3] were determined by the methods of Fiske and SubbaRow (5), serum chloride by the method of Van Slyke (6),[2] and urinary and homogenate chloride by the method of Wilson and Ball (7).

Blood glucose concentration was determined by the method of Nelson (8),[2] and quantitative urine glucose by the method of Benedict (9).

Urinary uric acid was determined by the method of Archibald as described by Forsham (10), and urinary creatine and creatinine by the method of Folin (11).

The ketosteroid and formaldehydogenic steroid determinations were carried out in the laboratory of Dr. Konrad Dobriner and represent values obtained from crude neutral fractions of urine (12). Urinary FSH was determined by the method of Klinefelter, *et al.* (13).

A muscle biopsy specimen was taken from the pectoral fold, under local anesthesia. Two ligatures placed above and below the specimen to be removed excluded the novocain solution from it. The muscle was analyzed in the same manner as previously described for the homogenates, except that the molarities of the $AgNO_3$ and NH_4SCN reagents in the chloride determination were those recommended by Lowry and Hastings (14).

Sodium and bromide spaces were determined by dilution techniques (15),[4] four, and five and one-half hours after injection.

The concentrations of sodium, potassium, and chloride per liter of packed erythrocytes were computed, using the formula (with K as an example):

$$\frac{K_c = K_b - (1 - V_c) \times K_s}{V_c}$$

where K_c, K_b and K_s are the concentrations in erythrocytes, whole blood, and serum respectively, in milliequivalents per liter and V_c is the hematocrit reading expressed as a fraction of 1.

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[2] These determinations were made in the Clinical Biochemistry Laboratory.

[3] Urinary inorganic phosphorus is only 5 per cent lower than total phosphorus in protein-free urines.

[4] The assistance of Drs. Leon Hellman and Eugene Y. Berger, respectively, in carrying out these determinations is gratefully acknowledged.

Adrenocorticotropic hormone (ACTH)[5] was administered intramuscularly in a 5 per cent glucose solution in four divided doses daily. Potassium chloride was administered orally as a 3 per cent aqueous solution.

CLINICAL OBSERVATIONS

The patient was a 43-year-old Negro woman in whom, since the age of 34, there had developed amenorrhea, hypertension, generalized weakness, increased facial hair, diminution of scalp hair, increased fat around the face, neck and abdomen, increased glove and shoe sizes, a gradual weight gain of about 50 pounds, mild diabetes controlled with 25 units of protamine zinc insulin per day, and blurred vision with restriction of the lateral visual fields.

Physical examination revealed an obese woman with bitemporal hemianopsia, "moon facies," coarse facial hair, wide fingers and toes, numerous infected sebaceous cysts, a blood pressure of 166/110 mm. of Hg, and striae over the buttocks and hips.

X-ray examination of the skull showed destruction of the posterior clinoids of the sella turcica, slight mandibular prognathism and tufting of the terminal phalanges of the hands and feet.

Following a metabolic study (to be described subsequently), the patient underwent a craniotomy with partial removal of an acidophilic adenoma, by Dr. Bronson S. Ray. Postoperatively, she remained weak, gradually went downhill, became stuporous, had convulsions and died following a second craniotomy (six weeks after the first) which revealed a subdural empyema.

Autopsy showed an acidophilic adenoma, completely replacing the anterior pituitary. Microscopic examination of the tumor revealed a preponderance of eosinophilic tumor cells with an admixture of abundant chromophobe cells and a few basophil cells. Isolated basophil cells showed a homogeneous, hyaline cytoplasm.[6] The combined weight of the adrenals was 26 Gm. They showed numerous superficial nodules which proved to be hyperplastic adrenal cortical tissue. There was moderate osteoporosis of the spine and the skin was atrophic. The pancreatic islet tissue appeared normal. There was no visceromegaly. The subdural empyema had developed by extension from an infected sphenoid sinus.

Laboratory studies, preoperatively, revealed a normal blood picture with no polycythemia and a normal differential leucocyte count. The circulating eosinophils were 19 per cu.mm. Urinalysis showed glycosuria. Serum chemistry showed metabolic alkalosis, hypopotassemia (1.72–3.24 mEq./L), hypochloremia (85.2–96.4 mEq./L), hypophosphatemia (1.3–2.6 mg.percent), hyperglycemia (290–360 mg.percent) and hypercholesterolemia (364–432 mg.percent). The serum alkaline phosphatase was normal.

An analysis of skeletal muscle revealed the following contents per kilogram of dry, fat-free solids: K = 314.0 mEq., Na = 236.8 mEq., Cl = 152.9 mEq., P = 5.21 Gm. and

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[5] Obtained through the courtesy of Dr. John R. Mote, Armour and Co., Chicago, Illinois.

[6] We are indebted to Dr. A. A. Koneff, University of California, Berkeley, California, and to Dr. Arthur Allen of this hospital, who prepared specially stained sections from pituitary tissue removed at the time of operation and at autopsy. Basophil cells, originally thought to be absent, were clearly demonstrated, some with changes closely resembling those described by Crooke in Cushing's syndrome (16).

Table 1. Metabolic Data

Feriod	No.	Days	Water intake* (cc.)	Urine vol. (cc.)	Calcium† (mg./24 hrs.)			Phosphorus† (mg./24 hrs.)			Sodium† (mEq./24 hrs.)			Chloride† (mEq./24 hrs.)		Nitrogen† (Gm. /24 hrs.)			Potassium† (mEq./ 24 hrs.)			Urine glucose (Gm./24 hrs.)	Creatine (Gm./24 hrs.)	Creatinine (Gm./24 hrs.)
					Urine	Feces	Bal.	Urine	Feces	Bal.	Urine	Feces	Bal.	Urine	Bal.	Urine	Feces	Bal.	Urine	Feces	Bal.			
Control	1	3	4300	5390	247	135	−125	525	315	+136	64.1	3.7	+ 33.2	75.2	+ 25.5	10.90	1.51	+1.59	56.3	11.8	+ 11.4	103	0.52	1.36
	2	3	4210	5750	293	135	−171	621	315	+ 40	98.6	3.7	− 1.3	106.5	− 5.8	12.36	1.51	+0.13	65.9	11.8	+ 1.8	103	0.69	1.15
	3	3	4130	5540	259	233	−235	558	500	− 82	79.0	8.7	+ 13.3	106.4	− 5.7	11.85	2.29	−0.14	58.1	21.7	− 0.3	90	0.44	1.21
	4	1	4200	5720	243	233	−219	533	500	− 57	66.4	8.7	+ 25.9	64.2	+ 36.5	11.23	2.29	+0.48	59.6	21.7	− 1.8	114	0.69	1.14
		1	5960	6550	302	233	−278	548	500	− 72	87.2	8.7	+ 5.1	89.1	+ 11.6	12.53	2.29	−0.82	69.1	21.7	− 11.3	110	0.66	1.31
		1	4975	6235	261	233	−237	533	500	− 57	50.7	8.7	+ 41.6	59.9	+ 40.8	11.88	2.29	−0.17	56.7	21.7	+ 1.1	97	0.57	1.31
mg. ACTH 15	5	1	3425	3950	178	136	− 57	577	371	+ 28	40.6	5.2	+ 55.2	47.3	+ 53.4	11.67	1.80	+0.53	56.9	17.5	+ 5.1	148	0.47	1.11
45		1	4800	4660	325	136	−204	820	371	−215	132.0	5.2	− 36.2	158.4	− 57.7	12.97	1.80	−0.77	80.2	17.5	− 18.2	103	0.61	1.26
50		1	3000	5140	326	136	−205	1070	371	−465	168.0	5.2	− 72.2	194.7	− 94.0	13.95	1.80	−1.75	95.5	17.5	− 33.5	115	1.03	1.13
50	6	1	2750	4220	207	136	− 86	1075	371	−470	44.6	5.2	+ 51.2	57.7	+ 43.0	14.56	1.80	−2.36	63.2	17.5	− 1.2	81	0.72	1.56
50		1	2400	4260	233	136	−112	997	371	−392	42.1	5.2	+ 53.7	60.8	+ 39.9	15.83	1.80	−3.63	64.9	17.5	− 2.9	134	1.15	1.24
50		1	3000	4400	258	136	−137	916	371	−311	61.3	5.2	+ 34.5	89.3	+ 11.4	16.72	1.80	−4.52	69.9	17.5	− 7.9	150	1.10	1.19
50	7	1	2400	4150	232	204	−179	818	538	−380	42.6	2.2	+ 56.2	67.3	+ 33.4	15.69	1.80	−3.94	63.4	20.3	− 4.2	208	1.16	1.16
100		1	2400	4050	281	204	−228	911	538	−473	90.0	2.2	+ 8.8	111.0	− 10.3	16.75	1.80	−4.55	76.1	20.3	− 16.9	253	1.13	1.05
100		1	825	3550	223	204	−170	952	538	−514	61.9	2.2	+ 36.9	76.5	+ 24.2	16.73	1.80	−4.53	64.8	20.3	− 5.6	231	1.12	1.21
100	8	1	1375	3950	272	204	−219	935	538	−497	85.6	2.2	+ 13.2	109.2	− 8.5	18.85	1.80	−6.65	78.6	20.3	− 19.4	263	1.11	1.11
100		1	2625	4250	296	204	−243	1052	538	−614	147.0	2.2	− 48.2	164.0	− 63.3	19.05	1.80	−6.85	96.1	20.3	− 36.9	261	1.11	1.19
100		1	2850	4700	300	204	−247	996	538	−558	160.0	2.2	− 61.2	164.6	− 63.9	19.50	1.80	−7.30	106.0	20.3	− 46.8	290	1.17	1.13
Control	9	1	1800	3950	265	193	−201	769	436	−229	93.5	4.5	+ 3.0	114.8	− 14.1	17.98	1.71	−5.69	78.2	15.5	− 14.2	234	1.11	1.03
		1	2400	3525	173	193	−109	613	436	− 73	32.9	4.5	+ 63.6	50.3	+ 50.4	15.71	1.71	−3.42	50.6	15.5	+ 13.4	215	1.12	1.09
		1	2025	3400	213	193	−149	461	436	+ 79	67.5	4.5	+ 29.0	85.0	+ 15.7	14.31	1.71	−2.02	51.9	15.5	+ 12.1	211	1.01	0.95
	10	1	1800	2780	144	193	− 80	304	436	+236	35.9	4.5	+ 60.6	39.2	+ 61.5	11.26	1.71	+1.03	37.3	15.5	+ 26.7	164	1.03	0.89
		1	2400	2725	113	193	− 49	251	436	+289	18.8	4.5	+ 77.7	19.5	+ 81.2	9.53	1.71	+2.76	34.9	15.5	+ 29.1	156	1.12	0.87
		1	2600	3300	139	193	− 75	270	436	+270	26.9	4.5	+ 69.6	24.1	+ 76.6	9.89	1.71	+2.40	46.1	15.5	+ 17.9	186	1.15	0.92
KCl‡	11	1	2680	3750	125	152	− 20	246	351	+379	45.3	9.8	+ 45.9	40.4	+220.3	8.68	1.71	+3.61	66.5	21.9	+151.1	179	1.20	0.90
		1	2050	3550	174	152	− 69	228	351	+397	74.2	9.8	+ 17.0	92.3	+168.4	7.99	1.71	+4.30	178.0	21.9	+ 39.6	141	1.21	0.92
		1	2850	4520	226	152	−121	204	351	+421	118.0	9.8	− 26.8	208.6	+ 52.1	7.76	1.71	+4.53	176.0	21.9	+ 41.6	142	1.13	0.90
	12	1	2400	4040	269	152	−164	295	351	+330	165.0	9.8	− 63.8	290.5	− 29.8	8.29	1.71	+4.00	193.0	21.9	+ 24.6	110	0.48	0.97
		1	2200	4550	248	152	−143	375	351	+250	158.0	9.8	− 66.8	291.2	− 30.5	7.66	1.71	+4.63	188.0	21.9	+ 29.6	120	0.46	0.91
		1	3600	6090	340	152	−235	433	351	+192	251.0	9.8	−159.8	399.1	−138.4	7.00	1.71	+5.29	201.0	21.9	+ 16.6	168	0.55	0.91

* Exclusive of diet water, which was constant.

† The intakes per 24 hours were: Ca 257 mg.; P 976 mg.; Na 101 mEq.; Cl 100.7 mEq.; N 14 Gm.; K 79.5 mEq.

‡ K 160 mEq. and Cl 160 mEq. added to dietary intake.

N = 134.8 Gm.[7] Calculations derived from these figures demonstrated that the extracellular water content of muscle had been increased over normal by a factor of 1.8, at the expense of intracellular water. Analyses of erythrocytes showed the following concentrations per liter of packed cells: K = 88.0 mEq., Na = 28.5 mEq. and Cl = 49.7 mEq.[7]

The exchangeable sodium and chloride contents of the body, estimated by dilution techniques and expressed per unit of body weight, were: Na = 44.1 mEq./Kg. and Cl = 32.3 mEq./Kg.[8]

Increased sodium and chloride contents were thus demonstrated in skeletal muscle and in the body as a whole.

An electrocardiogram showed depressed ST segments and flat T waves consistent with potassium deficiency.

The basal metabolic rate and uptake and excretion of radioactive iodine (I^{131}) were within normal limits.

Urinary assay for FSH was negative for 6.5 mouse units. Urinary ketosteroids were 33.8 mg. per 24 hrs. (normal range for adult females: 5 to 15 mg. per 24 hours), and the formaldehydogenic steroids were 12.4 mg. per 24 hours (normal: 0.5 to 1.5 mg. per 24 hours). A bioassay of serum by Dr. A. Albert, before ACTH administration, demonstrated the presence of adrenocorticotropic activity.[9]

Following partial removal of the pituitary adenoma, the serum carbon dioxide content remained slightly elevated (31 to 32 m-mol./L) and the serum phosphorus low (1.3 to 2.1 mg./100 cc.). The hypercholesterolemia was not corrected. There was a marked reduction in urinary glucose excretion, but the fasting hyperglycemia persisted. The serum chloride and pH values returned to essentially normal levels, however. An adrenocorticotropic substance could no longer be demonstrated in the blood postoperatively and there was a marked reduction in urinary formaldehydogenic steroids.

METABOLIC STUDIES

Metabolic studies were undertaken in order: 1) to determine whether, and in what manner, an already hyperactive adrenal cortex would respond to stimulation by administered ACTH, 2) to measure the extent of the potassium deficit, and 3) to observe what metabolic and hormonal changes would ensue following removal of the pituitary tumor.

The patient was given a constant, low-calcium intake. After a twelve-day control period, ACTH in doses of 15 to 100 mg. per day[10] (Table 1) was administered for twelve days and, following a six-day recovery period, potassium chloride was given, 12 Gm. per day for six days. The data

[7] Mean "normal" values in skeletal muscle from 5 patients without evidence of electrolyte disturbance: K = 400.7 mEq., Na = 141.0 mEq., Cl = 97.4 mEq., P = 7.90 Gm. and N = 144.7 Gm.

Mean values in erythrocytes from 5 healthy adults: K = 100.4 mEq., Na = 18.1 mEq. and Cl = 54.1 mEq.

[8] The mean values in 5 healthy adults were: Na = 38.5 mEq./Kg. and Cl = 28.9 mEq./Kg.

[9] By this method, adrenocorticotropic activity is usually demonstrable only in the blood of patients with adrenal insufficiency (17).

[10] Equivalent to Armour Standard La-1-A.

on the intake, output, and balances of nitrogen and the electrolytes are found in Table 1, as well as the urinary excretion of glucose, creatine and creatinine.

During the control period the patient was in approximate nitrogen balance. After ACTH administration, a stepwise increase in nitrogen loss occurred. On doubling the dose of ACTH, there was a further increase of nitrogen loss to 7.3 Gm. on the twelfth day. When ACTH was stopped, the loss diminished rapidly until a markedly positive balance resulted. Increasing retention of nitrogen occurred during the period of potassium chloride administration.

The serum phosphorus levels were persistently low (1.3 to 2.6 mg. per cent) and stand in contrast to the slight elevation frequently observed in acromegaly (18).

During the control period more phosphorus was retained than would be predicted from the nitrogen and calcium balances, suggesting repair of a tissue deficit. It will be recalled that this patient's muscle phosphorus content was 34 per cent below the mean "normal." During the period when ACTH was given, there was a loss of phosphorus in excess of that expected. When ACTH was stopped, the phosphorus loss diminished rapidly, resulting in a positive balance. Postoperatively, the hypophosphatemia persisted.

The calcium balance was negative during the control period and was not appreciably changed during the period of ACTH administration.

Low serum potassium levels (1.72 to 3.24 mEq./L) were observed before and during administration of ACTH. The serum pH during these periods varied between 7.51 and 7.59 and the carbon dioxide content between 29.1 and 35.0 m-mol/L, indicating metabolic alkalosis. During the control period there was approximate potassium balance. When ACTH was first administered, and again after the dose was doubled, there were losses of potassium in excess of those expected from the nitrogen loss. When ACTH was discontinued, there was retention of potassium in excess of that expected, suggesting repair of a tissue potassium deficit. It will be recalled that both muscle and erythrocyte potassium contents were below the mean "normal." When potassium chloride was administered, a large retention of potassium resulted which, however, diminished gradually over the ensuing six days. Calculated intracellular balances show a moderate potassium loss during administration of ACTH and a marked retention when potassium chloride was given. A total of 307.1 mEq. of potassium was retained intracellularly after ACTH was stopped. The serum potassium, pH and carbon dioxide content returned to normal levels following administration of potassium chloride, suggesting that the tissue deficit had been repaired.

There was hypochloremia (85.2 to 96.4 mEq./L) which persisted until potassium chloride was administered. During administration of ACTH, losses of both sodium and chloride occurred initially, followed by a period of retention. Losses of these ions recurred after the ACTH dose was doubled. During the entire ACTH period there was, however, a net retention of 91.9 mEq. of sodium and a net loss of 92.4 mEq. of chloride. When potassium chloride was administered, there was a loss of 254 mEq. of sodium and a retention of 242 mEq. of chloride. The sodium loss was accompanied by a diuresis and weight loss of 3.9 Kg.

The circulating eosinophil levels and urinary excretion of creatine, creatinine, glucose, and steroids are shown in Figure 1. During ACTH administration the circulating eosinophils dropped from 19 to 0 per cu. mm. There was a twofold rise in urinary creatine excretion. During the last three days of potassium chloride administration the urinary creatine dropped abruptly to control-period levels. An almost threefold increase in urinary glucose excretion occurred during ACTH administration and the urinary ketosteroids and formaldehydogenic steroids likewise showed approximately threefold increases. Postoperatively, as previously noted, there was a marked reduction in glycosuria in spite of persistent hyperglycemia and a greatly lowered excretion of urinary formaldehydogenic steroids.

DISCUSSION

The serum electrolyte and acid-base disturbances exhibited by this patient (*i.e.*, hypopotassemia, hypochloremia, and metabolic alkalosis) have been encountered frequently in patients with hyperactive adrenal cortical states, whether spontaneous (19, 20) or induced by ACTH or cortisone (15, 21). Analyses of muscle from such patients have shown that there is an associated intracellular potassium deficit (22, 23). Administration of adequate quantities of potassium chloride will correct the serum electrolyte and acid-base disturbances and repair the tissue deficit (21, 22, 24). The patient described in this paper unquestionably had a marked tissue potassium deficit, which was increased by administration of ACTH. The magnitude of the intracellular deficit, at the time ACTH was stopped, is indicated by the amount of potassium which was retained in excess of that expected from the behavior of the nitrogen during the recovery and the potassium-chloride periods, namely 307.1 mEq. (4.8 mEq./Kg.). This is equivalent to about 15 per cent of the total exchangeable body potassium in "well" female patients (25), or 10 per cent of that found in normal males (26).

Whether the increased retention of nitrogen observed during the administration of potassium chloride was a result of the latter is not clear,

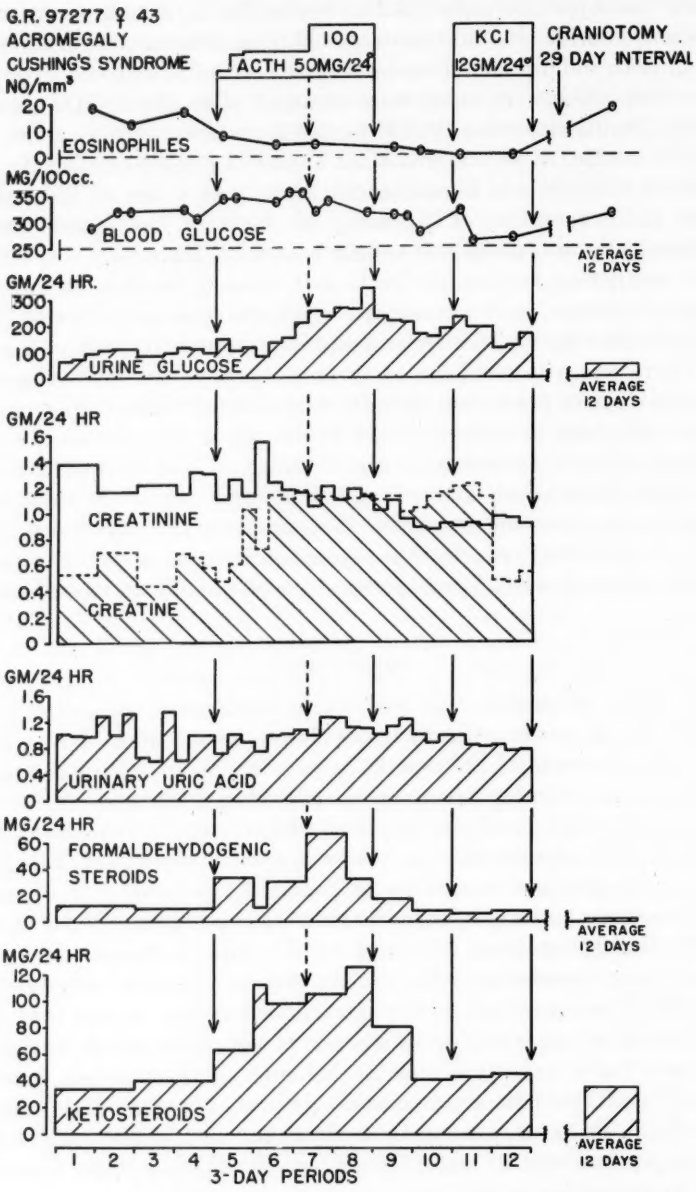

FIG. 1. Effects of ACTH and potassium chloride on circulating eosinophils and blood glucose; and on urinary glucose, creatinine, creatine, uric acid, formaldehydogenic steroids and ketosteroids.

since the urinary nitrogen had been falling anyway after ACTH was stopped. Kepler *et al.*, however, made a similar observation in a patient with an adrenal cortical tumor and Cushing's syndrome, who was given potassium chloride (22). We have observed a decrease in the negative nitrogen balance upon administration of potassium chloride to a patient who was receiving ACTH (27) and Bennett and Whitney have more recently shown that high intakes of potassium chloride will abolish the increased urinary nitrogen excretion produced by ACTH in rats (28).

There is suggestive evidence then that the nitrogen loss observed in hyperadrenocortical states may in part be related to potassium deficit. On the other hand, we have shown that potassium deficit in the human with normal adrenal cortical function does not result in nitrogen loss (29).

The abrupt drop of urinary creatine to control levels, which was observed when potassium chloride was administered, leads one to speculate whether potassium deficit may inhibit the synthesis of phosphocreatine and thus result in increased excretion of creatine.

There is also some experimental evidence that potassium deficit may be related to impairment of glucose metabolism. Gardner and his co-workers (30) have demonstrated the development of hyperglycemia and a diabetic type of glucose tolerance curve in rats maintained on potassium-deficient diets for prolonged periods; and Bennett and Whitney have shown that a high potassium diet given to partially depancreatized diabetic rats will reduce the increased glycosuria produced by ACTH (28). There is a suggestion from our data (Fig. 1) that administration of potassium may have resulted in slight reductions in hyperglycemia and glycosuria.

The occurrence of a tissue phosphorus deficit in hyperadrenocorticism has not received much attention. We have observed hypophosphatemia and a muscle phosphorus deficit in patients treated with ACTH or cortisone (29, 31).

The losses of intracellular phosphorus and potassium, which occurred when ACTH was given, contrast with the findings of Bartter *et al.* (32). They demonstrated apparent intracellular retention of these elements in several patients with panhypopituitarism who were given ACTH. The retention was attributed to glycogen deposition.

The clinical implications of tissue phosphorus deficit are, however, not clear. One wonders if impaired phosphorylating mechanisms, resulting from phosphorus deficit, might not in part account for the reduced glucose utilization (33) or reduced renal tubular reabsorption of glucose (34, 35) which are believed to occur in hyperadrenocortical states. Following partial removal of the patient's pituitary tumor, her urinary glucose excretion fell markedly, while the blood sugar remained elevated, suggesting an increase in renal tubular glucose reabsorption. No evidence was obtained,

however, to indicate that the phosphorus deficit had been repaired. A possible relationship of potassium deficit to the glycosuria has been discussed.

The paradoxical losses of sodium and chloride observed during the period of ACTH administration are not surprising in view of observations that DCA (36) or ACTH (37) given to patients with Cushing's syndrome or congenital adrenal hyperplasia, respectively, results in a loss, rather than a retention, of sodium and chloride. Furthermore, Forsham *et al.* (38) have shown that cortisone, administered to patients with Addison's disease who had been receiving DCA, likewise results in losses rather than retentions, of sodium and chloride. Bartter *et al.* (32) have observed losses of sodium and chloride in patients with panhypopituitarism who were given ACTH. The losses occurred on the first day and after one to two weeks of hormone administration. The early loss was attributed to pitressin in the ACTH preparations, since a similar loss was observed with pitressin alone. Late losses of sodium and chloride have been found, however, in some patients receiving cortisone (21), thus excluding in these instances an effect from exogenous pitressin. It cannot be stated from our data what role a pitressin effect played in the losses of sodium and chloride observed. Weight curves constructed from our data do indicate, nevertheless, that there was water retention during the early part of the ACTH period, in excess of sodium or chloride retention.

Some of the evidence suggests that the acidophilic tumor produced ACTH, as well as growth hormone: 1) adrenocorticotropic activity in the blood was demonstrated preoperatively, but not after partial removal of the tumor; 2) postoperatively, there was a marked reduction in urinary formaldehydogenic steroids; and 3) there was also postoperatively a marked reduction in glucose excretion, perhaps due to decreased "S" (sugar) hormone or "glucocorticoid" production. It would seem reasonable to attribute these changes to removal of acidophilic tumor cells, but it should be noted that some basophil cells were included in the excised portion of the tumor. ACTH production cannot, therefore, definitely be ascribed to the acidophilic tumor. It is of course possible that the adrenocortical hyperfunction resulted from growth hormone stimulation but no evidence was obtained which would bear on this problem.

Albright's contention that the basophilic cells of the pituitary produce FSH (39) is somewhat strengthened, since in this patient acidophilic cells were preponderant and urinary FSH was less than 6.5 mouse units per 24 hours.

The etiologic significance of the basophilic cell changes described by Crooke (16) in Cushing's syndrome is not further clarified by this study. Laqueur has recently demonstrated morphologic changes, similar to those

described by Crooke, in the basophilic pituitary cells of 5 patients who had received cortisone, thus strengthening the view that these alterations are the result, rather than the cause, of adrenocortical hyperfunction (40).

SUMMARY

Metabolic studies have been described in a Negro woman who had Cushing's syndrome and acromegaly associated with adrenal cortical hyperplasia and an acidophilic adenoma of the pituitary. The patient was given ACTH followed by potassium chloride and was then subjected to craniotomy for partial removal of the pituitary tumor.

A marked loss of nitrogen occurred during administration of ACTH. In the tissues there were pronounced phosphorus and potassium deficits, increased by ACTH. The potassium deficit, hypochloremia and metabolic alkalosis were corrected by administration of potassium chloride.

ACTH also resulted in a doubling of the creatine output and approximately threefold increases in urinary glucose, ketosteroid and formaldehydogenic steroid excretion.

The data suggest a possible relationship between the nitrogen loss, creatinuria, glycosuria and the occurrence of potassium deficit. The possibility that the phosphorus deficit was related to the patient's hyperglycemia and glycosuria is also discussed.

Increased extracellular fluid volume and increased sodium and chloride contents were demonstrated both by tissue analyses and dilution studies.

Losses, rather than retentions, of sodium and chloride were produced initially by ACTH.

The metabolic changes which have been described demonstrate that this patient's adrenal cortices, though hyperplastic, were capable of further increased function under stimulation by ACTH.

It is suggested, but not clearly established, that the pituitary tumor caused the adrenal hyperplasia. Its removal was followed by decreased adrenocorticotropic activity in the blood, and marked reductions in urinary glucose and formaldehydogenic steroid excretion.

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A COMPARISON OF THE 17-KETOSTEROID EXCRETION IN CUSHING'S SYNDROME ASSOCIATED WITH ADRENAL TUMOR AND WITH ADRENAL HYPERPLASIA*

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AN EXCESS of the gluconeogenic (sugar or "S") hormone of the adrenal is a *sine qua non* for the development of Cushing's syndrome. The gluconeogenic hormone is thought by many to be derived from the fascicular zone. To this hormone may be attributed the disordered sugar metabolism and the atrophy of skin, muscle, and bone. Whether the hypertension, edema, and changes in the blood electrolyte pattern (Kepler (1, 2), McQuarrie (3)) are due to the same or to a separate (DCA-like or sodium-retaining) hormone fortunately is not vital to the present argument. In any case, assays for gluconeogenic hormone in Cushing's syndrome should show an increase and they do.

The clinical manifestations of Cushing's syndrome are opposite in certain fundamental respects to those of the virilizing adrenal syndromes (4). The chief hormone responsible for the latter condition was thought by Blackman (5) to be derived from the reticular zone. The adrenal component of the 17-ketosteroid excretion is used as an index of the activity of this zone. It is obvious that the urinary 17-ketosteroids should be increased in the virilizing adrenal syndromes, and they are.

The two above mentioned adrenal cortical hormones appear to be functionally separate whether or not they can be assigned to the anatomic zones.

What would one expect the excretion of 17-ketosteroids to be in Cushing's syndrome?

The pathologic changes in the adrenal in Cushing's syndrome may be divided into three types: (A) without tumor of the adrenal cortex, (B) with benign tumor of the adrenal cortex, and (C) with malignant tumor of the adrenal cortex.

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These studies were carried out on the Metabolic Ward. The expenses of the bed which was used were defrayed by Mr. Edward Mallinckrodt, Jr.

A. 17-Ketosteroid excretion in cases without tumor

The increase in gluconeogenic hormone in the absence of an adrenal tumor presumably results from an increased stimulation of both adrenal cortices by ACTH (adrenocorticotropic hormone). In most, but not all, of these cases adrenal hyperplasia is demonstrable. Since administration of ACTH to normal individuals results in an increase in all functions of the adrenal cortex (6, 7) and a rise in 17-ketosteroid excretion (*circa* 30 mg./24 hrs.), comparable rises would be expected in Cushing's syndrome due to adrenal hyperfunction secondary to overproduction of ACTH.

B. 17-Ketosteroid excretion in cases with benign tumor

With the combination of a benign adrenal tumor and Cushing's syndrome it is most likely that the tumor is derived from the gluconeogenic hormone-producing cells (fascicular zone?). If the tumor itself is not producing other 17-ketosteroid precursors as well (and most of the urinary 17-ketosteroid is normally derived from different cells (reticular zone?)) one would expect not only no rise in 17-ketosteroids but actually a fall. Thus one would anticipate that the excess gluconeogenic hormone from the tumor would increase the urinary 17-ketosteroids but slightly, while inhibiting the production of ACTH and hence the production of 17-ketosteroid percursors from the uninvolved adrenal tissue. This would explain the atrophy of the contralateral adrenal (8) in such cases and the extremely low levels of 17-ketosteroid excretion which are known to follow the removal of these tumors.

C. The 17-ketosteroid excretion in cases of malignant tumor

In the case of tumors consisting of undifferentiated cells one would expect unpredictable kinds and amounts of hormone production and hence unpredictable 17-ketosteroid excretions. From analogy with other malignant endocrine tumors, enormous amounts of hormone would not be surprising.

It must be realized that there may be transitional states. A malignant tumor which produces a very large amount of ketosteroid must at some stage have produced only a moderate amount. Furthermore, transition from group "A" to group "B" may occur with the following sequence of events: 1) hyperplasia, 2) multiple adenomata, 3) predominance and autonomy of one adenoma, and 4) regression of other adenomata and of hyperplasia.

Figure 1 represents in diagrammatic form the hormonal relationships in (a) the normal, (b) adrenal hyperplasia causing Cushing's syndrome, and (c) benign adrenal tumor causing Cushing's syndrome.

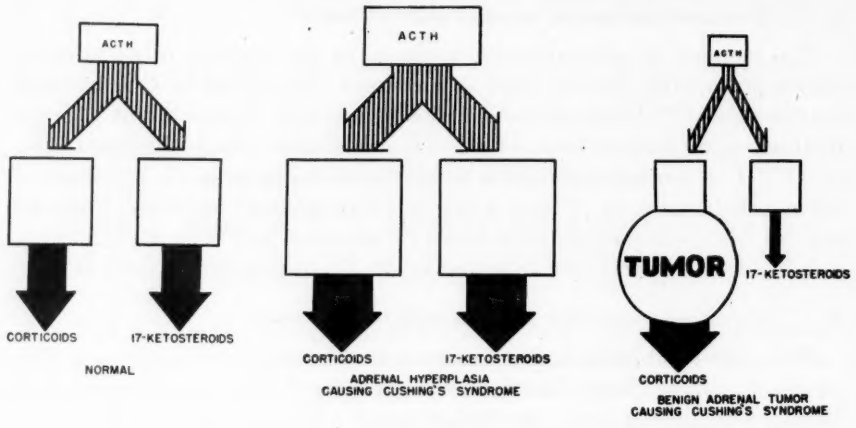

FIG. 1. The hormonal relationship in Cushing's syndrome. Each pair of squares represents the gluconeogenic hormone producing cells (left) and the androgen producing cells (right) of the adrenal cortex, respectively.

In summary, given the 17-ketosteroid excretion of a patient with Cushing's syndrome, one would guess that the findings on adrenal exploration would be:

with low excretion.....	benign tumor
with moderately increased excretion.....	(bilateral) hyperplasia
with markedly increased excretion.....	malignant tumor

RESULTS

The adrenals have been visualized either at operation or at post mortem in 24 cases of Cushing's syndrome at the Massachusetts General Hospital. Twelve cases without tumor are listed in Table 1. The average 17-ketosteroid excretion was 17.8 mg. per 24 hours and the deviation from the average normal value for sex and age (9) was +9 mg. per 24 hours.

Nine cases with benign adenoma are listed in Table 2. The average 17-ketosteroid excretion was 5.1 mg. per 24 hours and the average deviation from the normal value for sex and age was −1.6 mg.

Three cases of malignant adrenal tumor are listed in Table 3. The three values for urinary 17-ketosteroids were: 74 mg., 11.9 mg., and 19.9 mg. per 24 hours.

Additional cases, in which descriptions of the adrenals and the 17-ketosteroid excretion were available, were collected from other clinics and from the literature. These are listed in Tables 4 through 6. These data are, with few exceptions, consistent with the findings in our cases.

TABLE 1. THE 17-KETOSTEROID EXCRETION IN CASES OF CUSHING'S SYNDROME WITHOUT ADRENAL TUMOR (MASSACHUSETTS GENERAL HOSPITAL)

Patient	M.G.H. No.	Sex	Age	Adrenal findings	17-KS excretion (mg./24 hrs.)	Deviation from average for sex and age
C.D.	526033	M	31	Hyperplasia; bilateral biopsy	33.7	+18.7
L.B.	527071	F	37	Hyperplasia; bilateral biopsy	16.8	+10.8
I.G.	350260	F	13	Hyperplasia; bilateral biopsy	14.8	+10.8
B.A.	234190	F	40	Hyperplasia; autopsy	16.0	+10.0
R.B.	207693	F	47	Hyperplasia; both seen	18.0	+12.0
A.D.	499922	F	20	Hyperplasia; bilateral biopsy	18.3	+ 9.3
L.S.	511658	M	30	Hyperplasia; unilateral biopsy	21.2	+ 6.2
W.S.	598819	M	26	Hyperplasia; bilateral biopsy	19.0	+ 4.0
E.McL	397957	F	49	Hyperplasia; unilateral biopsy	14.9	+ 8.9
M.K.	511558	F	17	Hyperfunction; bilateral biopsy	9.2	+ 2.2
M.A.	93072	F	27	Hyperplasia; bilateral biopsy	19.1	+10.0
D.O'B	659364	F	33	Hyperplasia; unilateral biopsy	12.0	+ 5.5
				Average	*17.8*	*+ 9.0*

DISCUSSION

The surprisingly good agreement of these data with our train of reasoning leads us to carry the latter a few steps further. ACTH stimulates all the cells of the adrenal cortex. These (in a functional sense) comprise three groups: those producing gluconeogenic hormone, those producing androgen, and those producing DCA-like hormone. The separate identity of the last will not be questioned for the present. The hormonal product of a target gland usually inhibits production of the tropic hormone which stimulates it. If ACTH has three targets, any one of the three steroid hormones should inhibit its production. This lowered production of ACTH should be reflected in decreased production of the other two steroid hormones. Evidence that such cross inhibition occurs in the case of endogenous

TABLE 2. THE 17-KETOSTEROID EXCRETION IN CASES OF CUSHING'S SYNDROME WITH BENIGN ADRENAL TUMOR (MASSACHUSETTS GENERAL HOSPITAL)

Patient	M.G.H. No.	Sex	Age	17-KS excretion (mg./24 hrs.)	Deviation from average for sex and age
M.H.*	482838	F	33	4.4	−2.1
M.S.	484185	F	42	3.8	−2.2
B.A.	467276	F	33	7.5	+1.0
J.D.	610989	F	37	1.7	−4.3
S.W.	619603	F	33	5.0	−1.5
M.d'A	642624	F	36	2.3	−2.7
R.K.	387969	F	31	9.0	+1.5
M.C.	718895	F	30	6.9	− .6
R.B.	721778	F	30	5.5	−2.0
			Average	*5.1*	*−1.6*

* This patient had shown typical Cushing's syndrome followed by a partial remission. At operation an hyalinized tumor was found.

gluconeogenic hormone has just been presented. Furthermore, cortisone, although itself excreted in part as a 17-ketosteroid, except in very high dosage usually lowers a normal or high 17-ketosteroid excretion (10). Evidence that androgen (testosterone) can lower the production of gluconeogenic hormone has been presented elsewhere (11)(12). Evidence that DCA can do so is not yet adequate.

The hypotheses presented above, and the principle of crossed inhibition

TABLE 3. THE 17-KETOSTEROID EXCRETION IN CASES OF CUSHING'S SYNDROME WITH MALIGNANT ADRENAL TUMOR (MASSACHUSETTS GENERAL HOSPITAL)

Patient	M.G.H. No.	Sex	Age	Adrenal findings	17-KS excretion (mg./24 hrs.)	Deviation from average for sex and age
A.P.	110122	F	56	Ca. weighing 1 Kg.	74.0	+68.5
T.B.	365392	F	51	Ca. with metastases	11.9	+ 5.9
M.S.	347498	F	36	Ca. with metastases	19.9 (urine assayed after storing for 5 years)	+13.9

TABLE 4. THE 17-KETOSTEROID EXCRETION IN CASES OF CUSHING'S SYNDROME WITHOUT ADRENAL TUMOR (OTHER CLINICS)

Author or Clinic	Reference	Sex	Age	Adrenal findings	17-KS excretion (mg./24 hrs.)	Deviation from average for sex and age
Crooke, A. C. Callow, R. K.	(15)	F	12	Hyperfunction; ? hyperplasia	15.0	+12.0
McCullagh, E. P., Cleveland Clinic	(16)	M	11	"No gross abnormality;" (hyperfunction)	11.5	+ 8.5
McCullagh, E. P., Cleveland Clinic	(16)	F	39	Enlarged 2½ times, containing adenomas	26.1 (after removing one gland)	+20.1
McCullagh, E. P., Cleveland Clinic	(17)	F	26	Normal-sized left, and enlarged right	7.1	− 2.4
Wilkins, L., Baltimore	(18)	F	11	Hyperplasia	10.3	+ 7.3
Wilkins, L., Baltimore	(18)	M	13½	Hyperplasia	16.8	+12.8
Wilkins, L., Baltimore	(18)	M	13	Hyperplasia	18.8	+14.8
Wilkins, L., Baltimore	(18)	F	14	Hyperplasia	7.2	+ 2.2
Donahue, M. A.	(19)	F	8	"Nodular hyperplasia;" total wt. 10 Gm.	6.2	+ 4.2
Loeb, R. Perera, G., New York City	(20)	F	32	Right adrenal showed hyperplasia at operation	15.0	+ 8.5
Loeb, R. Perera, G., N.Y.C.	(20)	F	29	Hyperplasia. Had sympathectomy and 1 myocardial infarct	4.4	− 3.6
Loeb, R., Perera, G., N.Y.C.	(20)	M	33	Biopsy specimen of right adrenal "showed hyperplasia;" left "normal"	15.7	+ 1.7
Loeb, R. Perera, G., N.Y.C.	(20)	M	15	Hyperplasia; one explored	40.0	+34.0
Loeb, R. Perera, G., N.Y.C.	(20)	F	26	Hyperplasia right adrenal; left not explored	13.4	+ 3.4
Paschkis, H. *et al.*	(21)	F	36	Markedly enlarged adrenals containing "knoten"	31.0	+25.0
Bjerglund, C. J. Torgerson, O.	(22)	M	55	Hyperplasia	21.0	+12.0
Mason, E. H.	(16)	F	42	"No distinct abnormality"	22.1	+16.1
Thorne, G. Forsham, P., Boston	(23)	M	27	Both adrenals enlarged	31.3	+16.1
Parsons, W., New Orleans	(24)	F	22	Hyperplasia	4.4	− 5.1
Mayo Clinic	(1)	F	29	Hyperplasia of both adrenals; patient also had myxedema	2.1	− 5.9
Mayo Clinic	(1)	M	29	Subtotal adrenalectomy for bilateral hyperplasia	28.4	+13.4
Mayo Clinic	(1)	F	38	Hyperplasia with tumor	10.0	+ 4.0
Mayo Clinic	(25)	F	27	Wt. of both adrenals 15.2 Gm.	9.0	± 0
Mayo Clinic	(26)	16 other cases in females		In all, diagnosis proved by exploration or autopsy	18.3 (av.)	+10.9 (av.)
Mayo Clinic	(26)	5 other cases in males		"	28.3 (av.)	+20.6 (av.)
				Average	*18.2*	*+10.8*

TABLE 5. THE 17-KETOSTEROID EXCRETION IN CASES OF CUSHING'S SYNDROME WITH BENIGN ADRENAL TUMORS (OTHER CLINICS)

Author or Clinic	Reference	Sex	Age	Adrenal findings	17-KS excretion (mg./24 hrs.)	Deviation from average for sex and age
Mayo Clinic	(27)	F	32	"Cellular adenoma"	2.4	−4.1
Mayo Clinic	(26)	F	50	"Hyperplastic adenoma"	5.6	−0.2
Hamblen, E. C., Duke Univ., Durham	(28)	F	36	"Benign adenoma"	8.0	+2.0
Guterman, H. S., Chicago	(29)	F	29	"Benign adenoma"	2.8	−5.2
Loeb, R., Perera, G., New York City	(20)	F	30	Benign tumor	6.1	−0.9
McCullagh, E. P., Cleveland Clinic	(17)	F	18	"Relatively benign" tumor, weighing 140 Gm.	3.8	−4.2
McCullagh, E. P., Cleveland Clinic	(17)	F	42	Bilateral tumors, containing abundant fuchsinophil material	11.9	+5.9
Luetscher, J. A., San Francisco	(30)	F	25	Benign adenoma, 8 cm. in diameter	7.8	−2.2
Thorne, G. W. Forsham, P. H., Boston	(23)	F	38	Benign adenoma	6.9*	+0.9
				Average	*6.1*	*−0.9*

* Two years before, 17-ketosteroids were 9.8.

lead to certain therapeutic suggestions:

1. First consider the case of the patient with Cushing's syndrome due to bilateral hyperplasia of the adrenals. He or she has an excess of gluconeogenic hormone and of ACTH (see Fig. 1b). A subtotal resection is performed. He or she now lacks gluconeogenic hormone and produces more ACTH than ever. Cortisone may be indicated; ACTH is contraindicated.

2. Now consider the patient with Cushing's syndrome due to benign adenoma: she[1] has an excess of gluconeogenic hormone and a decreased production of ACTH, which has led to atrophy of the adrenal (see Fig. 1c). The adenoma is removed. She now not only lacks ACTH but gluconeogenic hormone and the other adrenal hormones as well. Cortisone is indicated for the immediate replacement of the gluconeogenic hormone; ACTH may be indicated to stimulate its endogenous production. The question arises—why is there not a rapid rise in endogenous ACTH?

[1] We have heard of no cases in males.

TABLE 6. THE 17-KETOSTEROID EXCRETION IN CASES OF CUSHING'S SYNDROME WITH MALIGNANT ADRENAL TUMOR (OTHER CLINICS)

Author or Clinic	Reference	Sex	Age	Adrenal findings	17-KS excretion (mg./24 hrs.)	Deviation from average for age and sex
Mayo Clinic	(27)	F	15 mos.	"Adenocarcinoma grade II"	4.1	+ 3.6
Mayo Clinic	(27)	F	40 mos.	Carcinoma, grade II	6.8	+ 6.3
Mayo Clinic	(31)	F	26	"Adenocarcinoma, grade II"	225.0	+215.0
Mayo Clinic	(27)	F	42	"Adenocarcinoma, grade III"	3.2	− 2.8
Mayo Clinic	(26)	F	15 mos.	Adenocarcinoma	3.0	+ 2.5
Mayo Clinic	(26)	F	17 mos.	"Adenocarcinoma, grade II"	1.1	+ 0.6
Mayo Clinic	(26)	F	16	Adrenal-like ovarian adenocarcinoma, grade II	54.6	+ 48.6
Mayo Clinic	(26)	F	53	"Adenocarcinoma, grade II"	649.0	+643.2
Mayo Clinic	(26)	F	41	Carcinoma, grade IV	45.0	+ 39.0
Dingemanse, E.	(32)	F	42	"Malignant hypernephroma"	350.0	344.0
Crooke, A. C. Callow, R. K.	(15)	M	2	Adrenal carcinoma	53.0	+ 52.4
Crooke, A. C. Callow, R. K.	(15)	F	6	Adrenal carcinoma	525.0	+524.0
Hamblen, E. C., Duke Univ.	(28)	F	37	Adrenal carcinoma with metastases	27.0	+ 21.0
Goldberg, M., San Francisco	(33)	F	39	Adrenal carcinoma	62.4	+ 56.4
				Average	*143.5*	*+139.3*

Apparently once ACTH is thoroughly inhibited it does not return rapidly. The 17-ketosteroid excretion of such patients is known to remain low for as long as a year. Moreover, as long as cortisone or ACTH treatment is continued, no return could be expected. A second question arises—is the continued failure of endogenous adrenal hormone production in the untreated cases due to inability of the atrophied adrenal to respond to ACTH? This is unlikely, since administration of ACTH to patients with panhypopituitarism usually causes a prompt rise in hormone production (7).

The principle of crossed inhibition applies to a third type of patient. She has virilism which may be due to hyperplasia of the reticular zone of the adrenal. Gluconeogenic hormone is, if anything, decreased while androgen production is increased. Cortisone inhibits the production of ACTH and hence of androgen (13) (14).

SUMMARY

I. Ninety cases of Cushing's syndrome (24 studied personally) have been collected in which the adrenals have been visualized and 17-ketosteroid assays made.

II. These cases have been classified into three groups, according to the adrenal pathologic changes: (A) without tumor, (B) with benign tumor, and (C) with malignant tumor.

III. The average 17-ketosteroid excretion of the three groups was: without tumor, 18.1; with benign tumor, 5.7; and with malignant tumor, 124.4 mg. per 24 hours.

IV. The pathologic physiology underlying these differences is discussed and certain therapeutic indications mentioned.

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EFFECTS OF LUTEOTROPIC DOSES OF CHORIONIC GONADOTROPIN IN WOMEN*

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THE first gonadotropic principle to be demonstrated clearly was that derived from human chorionic elements and present in the urine of pregnant women. Since its demonstration in 1927, innumerable studies have catalogued its effects in laboratory animals. There have been few well-controlled studies, however, in the normal human female. Browne and Venning (1) reported increased excretion of pregnandiol and delay of menstruation with relatively small doses of chorionic gonadotropin, although these observations were not confirmed by Brown and associates (2) when they used the same dosage of hormone. Brown and Bradbury (3) later demonstrated unequivocally the luteotropic effect of chorionic gonadotropin in the human. Using relatively large doses of the substance (made possible by more highly purified preparations), they induced pseudopregnancy in women, as evidenced by persistence of the corpus luteum, delay of menstruation, decidual changes in the endometrium and prolonged excretion of pregnandiol. Their results paralleled those of Hisaw (4) in the monkey. Since in the rat prolactin is known to function as luteotropin and is, indeed, necessary for the physiologic activity of corpora lutea, it is logical to ask whether the luteotropic action of chorionic gonadotropin in the human is in any way related to prolactin.

Effects of chorionic gonadotropin in women, other than luteotropic, have not been unequivocally demonstrated. It is known that in rodents chorionic gonadotropin acts synergistically with FSH and probably stimulates FSH release by the pituitary, but it is important to recall that chorionic gonadotropin is the product of the highly specialized human placenta and is a distinctly alien substance in the rodent. Whether it stimulates FSH release in the human cannot be answered simply by inference from its effects in lower animals. Suggestive evidence, however, has been offered by Claesson and coworkers (5), who administered crystalline hormone intravenously to amenorrheic women and observed the presence of one or more large ovarian follicles at subsequent laparotomy. This work deserves elaboration.

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It was the purpose of the present study to investigate further the luteotropic effects of chorionic gonadotropin[1] in women, with a variety of urinary hormone assays, combined with a morphologic study of its effects on the ovary and accessory structures.

MATERIAL AND METHODS

Six women with regular menstrual cycles were selected for study, and daily basal body temperatures were recorded both in a control cycle and throughout the experimental cycle. Administration of chorionic gonadotropin[1] was begun early in the postovulation period. As in the experiments of Brown and Bradbury (3), a standard daily dose of 10,000 I.U. of chorionic gonadotropin was given intramuscularly. Endometrial biopsy specimens were obtained in all women during both the control and experimental cycles, at a time when progestational endometrium was expected.

Urine was collected for hormone assays in each instance during the last half of the cycle. A modification of the Zimmermann reaction was employed to measure 17-ketosteroids. Biologic assay of glycogenic corticoids was performed by the method of Venning and associates (6). Urinary gonadotropins were measured by the method of Klinefelter and associates (7), and urinary prolactin was measured by the method of Coppedge and Segaloff (8).

Four of the 6 subjects each had an abdominal laparotomy during the artificially prolonged cycle, while under the influence of chorionic gonadotropin. In 2 instances the laparotomies were performed on the twenty-ninth and thirtieth days of the cycle, respectively. Although a greater indication of delay in menses would have been desirable in these 2 patients, their menstrual history indicated that they had cycles of approximately twenty-four and twenty-five days, respectively. In the other 2 subjects the operations were performed on the thirty-seventh and fortieth days of their prolonged cycles, respectively. In the 2 patients not operated on, chorionic gonadotropin was discontinued in one on the thirty-fifth day of the cycle, menstruation occurring three days later; and in the other, on the forty-second day of the cycle, menstruation occurring four days later.

RESULTS

Morphologic observations on specimens obtained during chorionic gonadotropin therapy. In all subjects treated with chorionic gonadotropin, the endometrium obtained either as a biopsy specimen or by hysterectomy showed a well developed decidual reaction, histologically indistinguishable from that of early pregnancy (Figs. 1 and 2). The decidual cells were con-

[1] Kindly supplied by Dr. E. C. Reifenstein, Jr., Ayerst, McKenna and Harrison, Ltd.

FIG. 1: Case 2. Endometrial tissue, showing decidual reaction toward surface and pronounced secretory activity of glands. (×30)

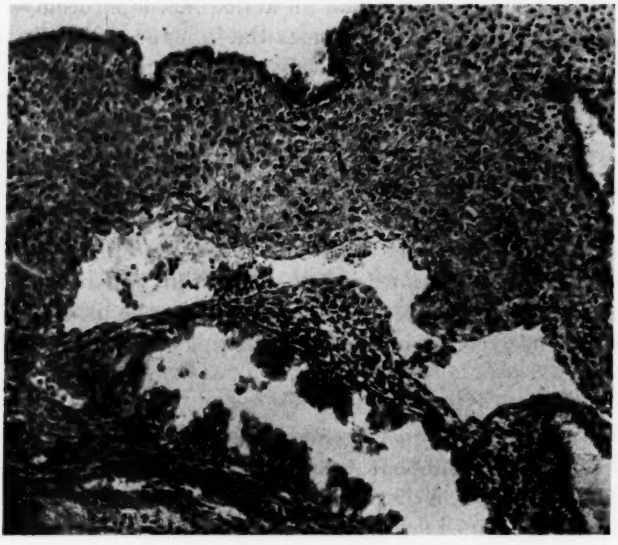

FIG. 2.: Case 2. Endometrial tissue showing well developed decidual reaction and secretory-phase endometrial gland. (×90)

siderably larger and more differentiated than those seen in the predecidual reaction of the endometrial stroma before menstruation (Fig. 3). In the specimens obtained by hysterectomy the decidual endometrium was thickened, soft and polypoid, and in 2 instances averaged 1 cm. in thickness.

The endometrial glands showed an exaggerated progesterone effect, with pronounced tortuosity, abundant secretion and foci of adenomatous hyperplasia (Fig. 4). In 2 instances changes in the secretory epithelium

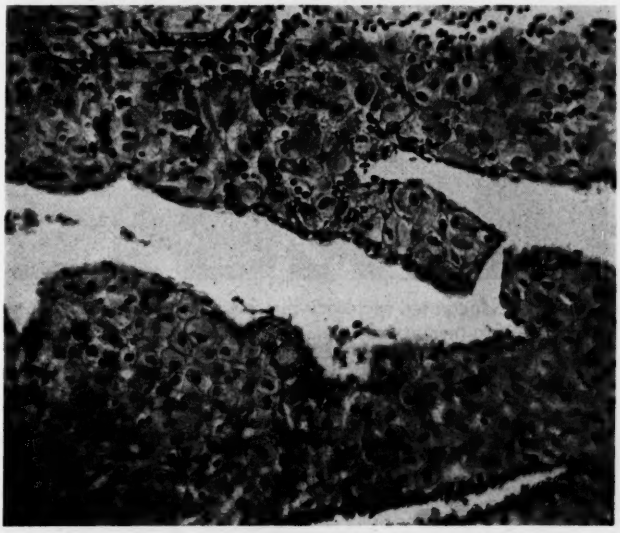

FIG. 3: Case 2. Endometrial tissue showing decidua identical with that of pregnancy. (×140)

were somewhat atypical and suggestive of carcinoma on casual examination. The glandular epithelium was heaped up, with confluence of the papillary infoldings, simulating solid aggregates of cells (Fig. 5). The nuclei in these areas were abnormally large, variable and hyperchromatic. These changes are considered the result of intense progesterone stimulation and unrelated to malignant transformation.

In the 4 instances in which ovarian tissue was examined histologically, conspicuous theca-luteinization of many of the follicles was present. Accumulations of theca-lutein cells were frequently seen in and about the scars of atretic follicles. A patchy decidual reaction simulating that occasionally seen in the ovaries during pregnancy was observed on the surface of the ovaries in 1 case (Fig. 6).

A study of the ovarian hilus cells (Leydig-like cells) indicated significant

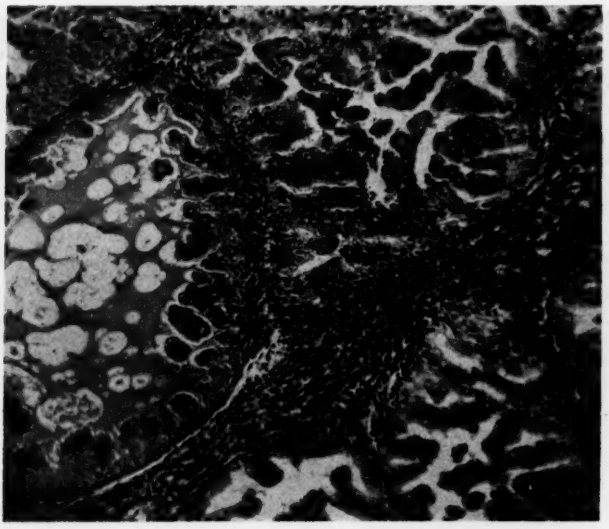

FIG. 4: Case 2. Exaggerated secretory-phase activity of endometrium, with papillary formations of glandular epithelium. (×90)

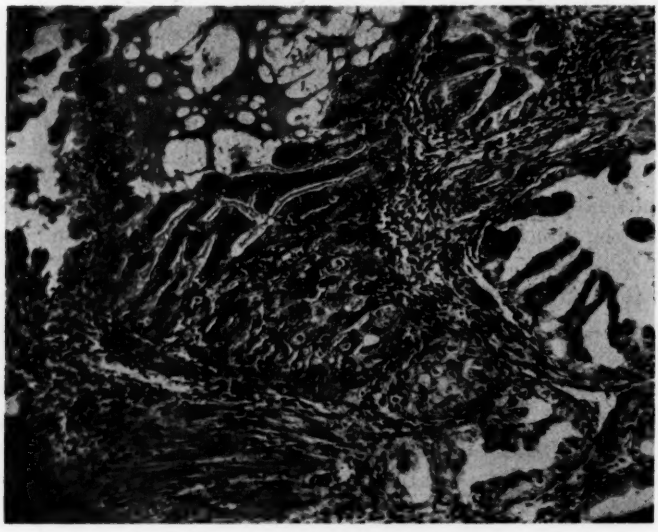

FIG. 5: Case 1. Exaggerated secretory-phase activity of endometrium with somewhat atypical glandular proliferation. (×90)

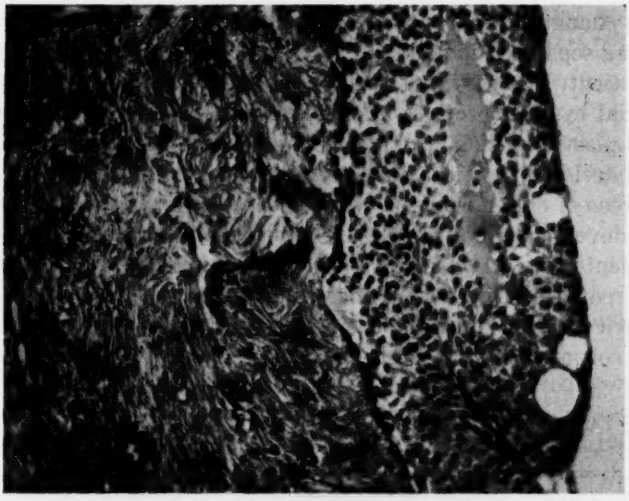

FIG. 6: Case 6. Decidual reaction of surface of ovary. (×140)

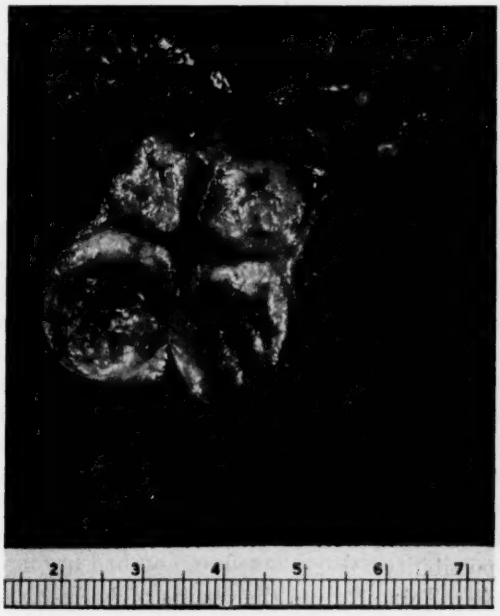

FIG. 7: Case 3. Ovary and tube, showing two well developed corpora lutea occupying the bulk of the ovary. (The ovary has been opened longitudinally.)

histologic changes in response to chorionic gonadotropin. This will be the subject of a separate report.

In 1 patient, an ovary was removed because of the presence of a large endometrial cyst. The cyst wall, on microscopic examination, was lined with hypoplastic epithelium but the subjacent endometrial stroma showed a distinct decidual reaction. Such a progestational change is not ordinarily seen in areas of endometriosis.

A well developed, and in some instances, cystic corpus luteum was seen in all patients who had a laparotomy. The surgical specimen from patient C. J. was remarkable because of the presence of two well developed corpora lutea which virtually replaced the entire substance of one ovary (Fig. 7). The two corpora lutea were clearly of the same cycle and histologically

TABLE 1. URINARY HORMONAL EXCRETION BEFORE AND DURING CHORIONIC GONADOTROPIN ADMINISTRATION*

Pt.	Age (yrs.)	Color	Sex	17-Ketosteroids (mg./24 hrs.)		Glycogenic corticoids (mg. Cpd. A/24 hrs.)		Gonadotropin (M.U./24 hrs.)		Prolactin (I.U./24 hrs.)	
				Before treatment	During treatment	Before treatment	During treatment	Before treatment	During treatment	Before treatment	During treatment
1 O.B.	33	C	F	5.4	9.4 (4)	0.46	0.21	>13.2<26.4	>576<1152		<50
2 E.J.	33	C	F	6.3	5.5 (4)	0.00	0.44	> 6.6<13.2	>480	<25	50-100
3 C.J.	39	C	F	5.1	17.4			>52.8<100	>480<576	0	88
4 C.G.	35	W	F	3.3	10.1 (4)	0.00	0.00	>13.2<26.4	>480	25-100	50-200
5 J.S.	23	W	F	8.3	14.5 (4)	0.50	0.41	> 6.6<13.2	>576	<25	25
6 W.H.	22	W	F	7.4	5.6 (4)	0.50	0.50	> 6.6<13.2	>480	<25	25

* All patients showed normal progestational endometrium in the cycle before and during therapy, and decidual endometrium in the cycle during therapy.

of active appearance, so that two ovulations must have occurred either simultaneously or within several days of each other. On the surface of the ovary overlying one of the corpora lutea was a small hillock with a central umbilicated depression; this indicates the site of rupture of the ovum and suggests that this corpus luteum was the younger of the two. Such double corpora lutea in the human are extremely rare, occurring if we may judge from the incidence of fraternal twins, once in about 112 ovulations. One cannot help speculating that the chorionic gonadotropin, administered soon after normal ovulation, may have induced an additional ovulation. The induction of multiple ovulations in animals by chorionic gonadotropin, as well as Claesson's work on follicle stimulation in the human, lends some weight to this possibility.

Hormone studies. Table 1 gives the results of hormone assays obtained in the cycle before therapy and in the cycle during therapy, in the 6 patients. In all there was a distinct decidual reaction in the cycle during therapy, an observation which indicates ovulatory cycles in all cases.

Likewise, all the urinary gonadotropic hormone assays before therapy fell within our average range for normally cycling women. In all instances there was the expected pronounced urinary excretion of chorionic gonadotropin in the cycle during therapy.

In all 5 of the 6 patients in whom assays were obtained before and during therapy there was a rise in the urinary prolactin during therapy. On the other hand, the values for urinary 17-ketosteroid excretion were not as consistent. Three patients showed a sustained increase in 17-ketosteroid excretion, whereas 2 showed a decrease. Essentially the same variability was seen in the assay of urinary glycogenic corticoids, 2 of the patients showing no change, 2 showing a decrease, and 1 showing an increase.

In each subject once or twice after cessation of treatment, serum was assayed for possible antihormones that might result from the use of chorionic gonadotropin. All these sera failed to show any measurable titer of antihormone.

DISCUSSION

Our results confirm the theory that chorionic gonadotropin, in the doses employed, is luteotropic. Onset of the menses was significantly delayed by use of this hormone. The longest period during which administration of the hormone was maintained was thirty-eight days; this was in patient J. S., who received injections from the twenty-fourth to the forty-second days of the cycle, and who menstruated on the forty-sixth day, four days after cessation of injections. In all patients the progestational endometrium was converted to a decidual one.

Of interest are the subjective responses of the patients to the therapeutic regimen. Four of the 6 subjects complained voluntarily of severe premenstrual symptoms consisting of headache, irritability, restlessness and depression, while receiving injections. Pain or tenderness of the breast, however, was not noted by any of the 6 women. These observations are of particular interest, since some investigators have attributed premenstrual tension to insufficient progesterone, and have in fact advocated treatment with chorionic gonadotropin.

The urinary steroid excretion varied from patient to patient. Since in women both 17-ketosteroids and glycogenic corticoids are measures solely of adrenal function, it is apparent that chorionic gonadotropin in the amounts employed had no significant consistent effect on the adrenals.

At the beginning of this study, it was thought that since prolactin had been demonstrated to act as luteotropin in rodents, the luteotropic effects of chorionic gonadotropin in women might be associated with a great increase in urinary lactogenic hormone. In the 5 patients in whom before-

and-after urinary prolactin assays were carried out, there was indeed an increase in this excretion. The increase appears to be a real one, since chorionic gonadotropin in the amounts excreted by these patients apparently does not affect the urinary lactogenic hormone assay employed. It is possible, then, that lactogenic hormone release is somehow involved in the luteotropic effect of chorionic gonadotropin in women.

SUMMARY

Six women with regular menstrual cycles have been treated with 10,000 I. U. daily of chorionic gonadotropin. Delay in the onset of the next menstrual period occurred in all and was accompanied by a decidual change in the endometrium. In all 6 patients large amounts of chorionic gonadotropin appeared in the urine. Urinary 17-ketosteroid and glycogenic corticoid excretions were variable, showing both increases and decreases. In the 5 patients in whom urinary prolactin excretions were obtained, there was an increase. In none of the patients did antihormones for chorionic gonadotropin develop. Other morphologic observations included the occurrence of unusually hyperplastic secretory glands of the endometrium in 2 instances; a decidual reaction of the ovary in 1, and a decidual reaction in an endometrial cyst of the ovary in another. One patient had two active corpora lutea present in one ovary.

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ENDOCRINE STUDIES IN AGING

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THE relation of glandular physiology to the aging process has been the basis of considerable theoretical speculation. The role of gonadal failure in senescence has for years been given prime consideration, but very few causal relationships have actually been established. The anterior pituitary, and more recently the adrenal cortex, have also received attention, but little is as yet known concerning the influence of hormones on the degenerative processes which accompany senility.

The present investigation was concerned with three objectives: One was to measure the mean values for the output of pituitary, gonadal, and adrenal cortical hormones in a group of old men and women, to determine possible sex differences, and to compare the values of the aged group with those encountered in young subjects. The second was to search for possible interrelationships between the changes in output of the three hormones as the aging process progressed. The third was to determine whether any correlation could be established between the functional activity of these glands as reflected by the assays, and the degree of aging as observed clinically in various organs and tissues.

MATERIAL

The group selected for study consisted of 35 men between the ages of 66 and 92 (mean 78) and 17 women between the ages of 69 and 85 years (mean 75). All resided at a Jewish home for the aged which is largely privately endowed. The residents of this home come from at least average economic status and all have had productive lives. Those chosen for this study were all essentially well and actively engaged in their daily tasks around the home. Included were 7 who had easily controlled diabetes, 4 with previously diagnosed and apparently successfully treated carcinoma, 1 with a mild degree of cardiac decompensation requiring digitalization, and 4 with low-grade diastolic hypertension of many years' duration. These were included with some reluctance; however, in the end it seemed evident that these disorders introduced no bias which significantly affected

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* Schering Research Fellow in Endocrinology.

the subjects' general physical or endocrine status. Two men died during the course of this survey, one of pneumonia and the other of rapidly progressing cardiac failure, but in both cases our studies preceded the terminal illness by several months.

METHODS

Each subject had had a recent thorough physical examination by a competent internist. In addition, the men were rechecked with special emphasis on the following clinical aspects considered to be of special importance in this study: 1) general appearance (tidiness of dress, body habitus, muscular development, evidence of weight loss), 2) appearance of skin (turgor, scaling, excoriations, "berry" angiomata, cutaneous hemorrhages, avitaminosis), 3) character of body hair (head, face, axillae, pubes), 4) character of genitalia (size, consistency), 5) mental state (memory, talkativeness, continuity), 6) status of palpable peripheral vessels (tenseness, tortuosity, beading) 7) estimation of adequacy of diet, 8) ophthalmologic examination for specific changes in cornea and lens (senile gutate keratitis, Hassall-Henle bodies, cataracts, arcus senilis), and 9) history of sexual development, fertility, persistence of potency and libido, and possible climacteric symptoms.

The women were examined less extensively, since participation was voluntary and full cooperation could otherwise not be obtained.

The following laboratory procedures were carried out: 1) assays of urinary total neutral 17-ketosteroids, formaldehydogenic corticoids (cortin) and gonadotropin, 2) roentgenograms of lumbar spine for estimation of degree of osteoporosis, 3) roentgenograms of lower leg for evaluation of large vessel calcification, 4) vaginal smears, for appraisal of ovarian function.

All x-ray films were exposed with the inclusion of a metal step gauge so that variations in film density due to differences in exposure and developing techniques could be evaluated and discounted.

An attempt was made to measure bone density, and thus osteoporosis, by a photoelectric densitometer. Multiple readings were taken over each lumbar vertebra in the lateral view and converted to a value expressed in terms of layers of the step gauge exposed on the same film. Adjacent soft tissue density was similarly measured and the result subtracted from the first value. We believed that we could thus obtain an objective estimation of bone density suitable for comparison. This method, however, failed to distinguish without overlap, a group of spine films of 7 young adults used as controls, from the films of the aged subjects. Further, a significantly greater degree of decalcification could not be demonstrated in the vertebrae of the aged women than in the men, in contrast to the

generally accepted belief that such a difference is present. Two experienced radiologists, without knowledge of the identity of the plates, were then asked to score the films of the entire group, according to the degree of osteoporosis present, using the usual criteria of increased radiolucence and prominence of vertical trabeculae. When this was done there was a high degree of agreement between the independent readings of the two radiologists. The normal young adults were invariably distinguished as having no osteoporosis and a definite difference between the sexes was evident in the senile subjects.

The apparent superiority of a subjective evaluation of osteoporosis over the densitometric method was unexpected. It is difficult to believe that the eye can distinguish differences in density which are not demonstrable by a photoelectric device. On the other hand, the eye is as much concerned with bone texture (trabeculation) as with absolute density. It might be questioned whether such a change in architecture is a valid measure of osteoporosis, which, in essence, implies loss of calcium. Nevertheless, it was believed best to accept the simple visual method for the quantification of osteoporotic change.

Urinary 17-ketosteroids were determined by the method of Holtorff and Koch (1), formaldehydogenic corticoids (cortin) by the procedure described by Corcoran and Page (2), and gonadotropin by the extraction procedure of Heller and Chandler (3) using mice as assay animals. For the latter, at least 8 and sometimes as many as 18 mice were used for each sample of urine. Levels of dosage were in multiples of 2 (*i.e.*, 4, 8, 16, etc.) mouse units per twenty-four hours. A unit consisted of the lowest dose of urine-equivalent which would produce a 100 per cent increase in uterine weight in 2 or more mice. All urine samples were preserved with toluene and every precaution was taken to insure an accurate collection over a 48-hour or 72-hour period.

RESULTS

Urinary gonadotropin

According to our laboratory findings, normal young adult males and females (avoiding the ovulatory peak) usually excrete between 8 and 32 m.u. per twenty-four hours. The mean value for 13 males and 11 females was 20 mouse units in each instance. One male and 2 females had values of 64 units, and 1 female excreted only 4 units. A value of 4 or 64 may therefore be considered borderline.

In the present series, of the 17 women, results in 6 (35 per cent) were positive at 128 m.u. and in 6 (35 per cent) at 64 m.u. Thus, 70 per cent of the assays in aged women exceeded the range of normal or were borderline-high. None was positive at titers beyond 128 m.u.; 5 (30 per cent) were

within the normal range; and none was below 8 units (Fig. 1). No significant correlation could be established between excretion level and age; however, if instead of chronologic age, the duration of time since the menopause was considered, a significant relationship became evident. The mean age at the menopause was 47.5 (St. E. 0.56) years, and the duration of time that lapsed until the present study ranged from 19 to 38 years with a mean of 27.1 years (St. E. 1.5). It was noted (Fig. 2) that

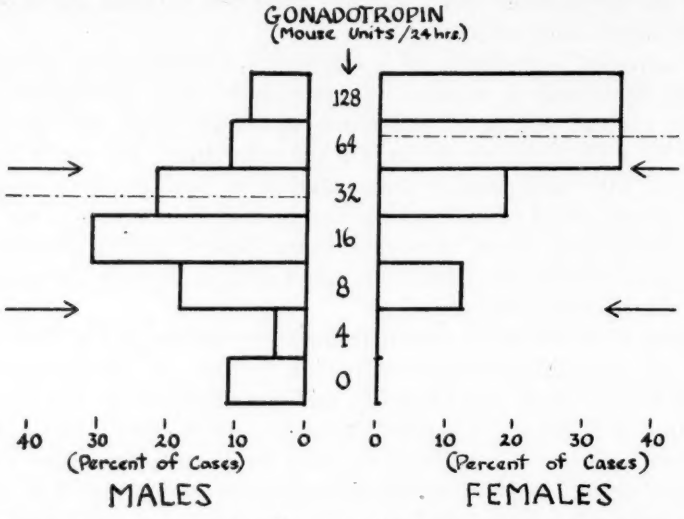

FIG. 1. *Gonadotropin excretion in the well-aged.* The arrows delimit the normal range in young adults. The broken lines represent the mean excretion levels found in the aged males and females.

women more than 25 years past the menopause had definitely lower excretion levels than those less than 25 years past. If the group is separated at the 24-year postmenopause mark, the two groups so formed are of equal number. The mean excretion for women less than 25 years past the menopause is 100 m.u. (St. E. 14.1), whereas for the group more than 25 years past, it is 51.5 m.u. (St. E. 12.4). The t value for the significance of the difference between these means is 2.6, so that the probability of this difference occurring by chance alone is 0.02. If the 30-year postmenopause dividing line is used, the difference is even more pronounced. Thus, for women 30 or less years past the menopause the mean gonadotropin excretion is 90.7 m.u. (St. E. 11.7) whereas for those more than 30 years past the value is 35.2 m.u. (St. E. 12.5). The t value here is 3.3; the p value is therefore considerably less than 0.01.

In the aged men, there were 3 who excreted 128 m.u. per twenty-four hours and 4 who excreted 64 units, a total of 7 (21 per cent) in whom the values were elevated. Twenty-two men (65 per cent) showed results within the normal range and 5 (14 per cent) had subnormal titers (Fig. 1). Of the latter group, 4 had no demonstrable gonadotropin activity at the 4 m.u. level, this being as low as the assay could be carried. Again no correlation could be established between gonadotropin excretion and age. This is not to imply that age does not affect the excretion. The effect, however, does not appear to be a simple one because within the group were individ-

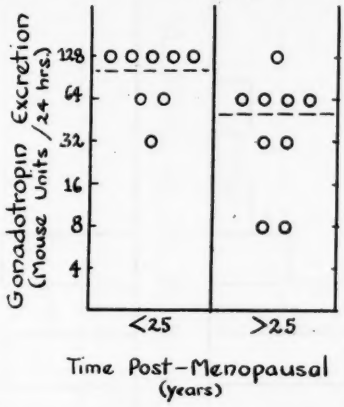

FIG. 2. *Effect of the time interval since the menopause on the gonadotropin excretion of aged women.* The broken lines represent the mean excretion levels for the two groups.

uals with very low values and others with higher values than are to be seen in young subjects.

As stated previously, vaginal smears were obtained in a certain number of women. Due to unwillingness on the part of some of the subjects, only 9 of the 17 women were so examined. In all 9 cases no fully cornified cells were identified, and each smear showed some typical deep (atrophy) cells. Among these 9 women, 7 showed a gonadotropin excretion of 64 units or more and 2, an excretion within the normal range. It would therefore appear from this sampling, that the failure to encounter elevated gonadotropin excretion in many of the subjects was not dependent on an unusually well preserved function of estrogen secretion.

Urinary 17-ketosteroids

In our laboratory, the normal range of the 17-ketosteroid excretion for men is 16 mg. to 34 mg. per twenty-four hours, with a mean of 22.6 (St. E.

1.05) (14 subjects). In women the range is 4.0 to 22.0, with a mean of 12.6 (St. E. 0.58) based on 30 observations.

For the 34 aged men on whom this determination was made, a mean of 9.2 mg. (St. E. 0.65) was found, with a range of 3.1 to 18.7 mg. Thus, the entire group of results was below the normal mean, and 31 (91 per cent)

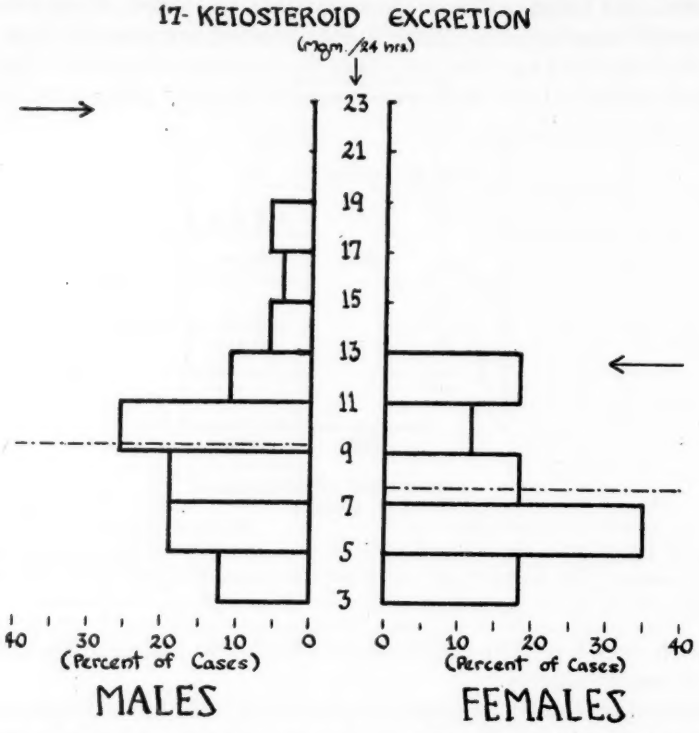

FIG. 3. *17-Ketosteroid excretion in the well-aged.* The arrows indicate the mean excretion levels for normal young adults. The broken lines indicate the means for the aged group.

were below the lower limit of normal (Fig. 3). When the group is broken down according to age, no significant difference can be found between the seventh, eighth, ninth, and tenth decades as regards excretion values or scatter (Fig. 4).

In the case of women, the mean excretion was 7.5 mg. (St. E. 0.69) and the range, 4.0 to 12.4 mg. per twenty-four hours. Thus again, the entire group of results was below the normal mean, but here all were within the normal range for women (Fig. 3). There was no variation with age (Fig. 4).

Thus, the aged men showed a mean ketosteroid excretion level approximately 40 per cent of that of young adult males, whereas the aged women showed a mean value 60 per cent of that of younger females. In young adults the mean value for females is 55 per cent of that of young males; in the aged group herein studied, this value was 80 per cent. Thus the

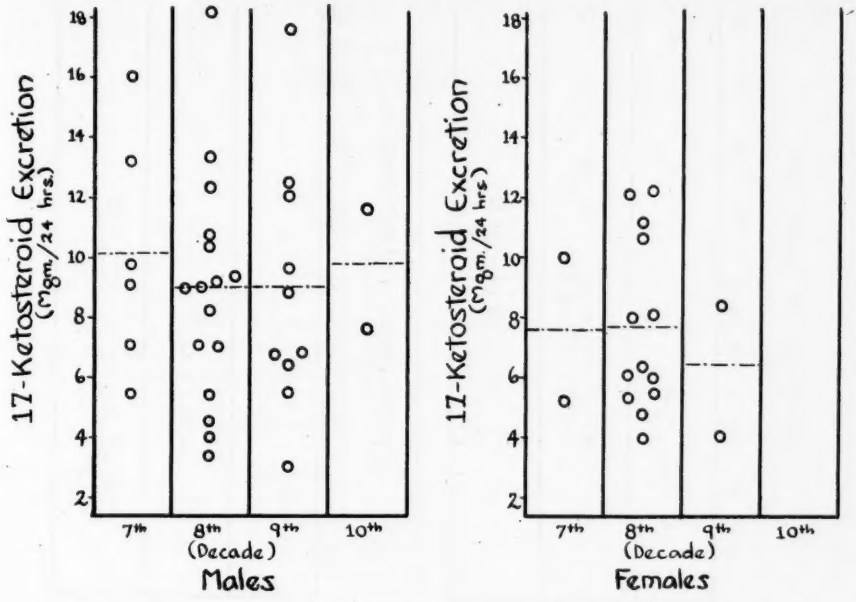

FIG. 4. *Effect of age (beyond the sixth decade) on 17-ketosteroid excretion.* The broken lines indicate the means for the various age and sex groups.

proportionate decline of androgen and androgen-like function with aging is found to be greater in males than in females.

There was no correlation between ketosteroid excretion and body weight, or body habitus, except for an anticipated decrease in excretion in those subjects who were 15 per cent or more below their ideal weight (4).

In both men and women, when 17-ketosteroid excretion was plotted against gonadotropin, although a suggestion of correlation was present (Fig. 5) this could not be proved significant by statistical analysis.

Urinary cortin

According to results in our laboratory, the mean cortin excretion in normal young adult males (10 subjects) is 0.34 mg. per twenty-four hours (St. E. 0.04), whereas for females (10 subjects) this value is 0.48 mg. per

twenty-four hours (St. E. 0.13). This difference between the sexes is not statistically significant ($t = 1.03$). The range of normal is comparable to that obtained by others using this method (5). Values for the group of aged men ranged from 0.1 mg. to 1.3 mg. per twenty-four hours, with a mean of 0.54 (St. E. 0.07). For women the range was less than 0.1 to 1.1 mg. per

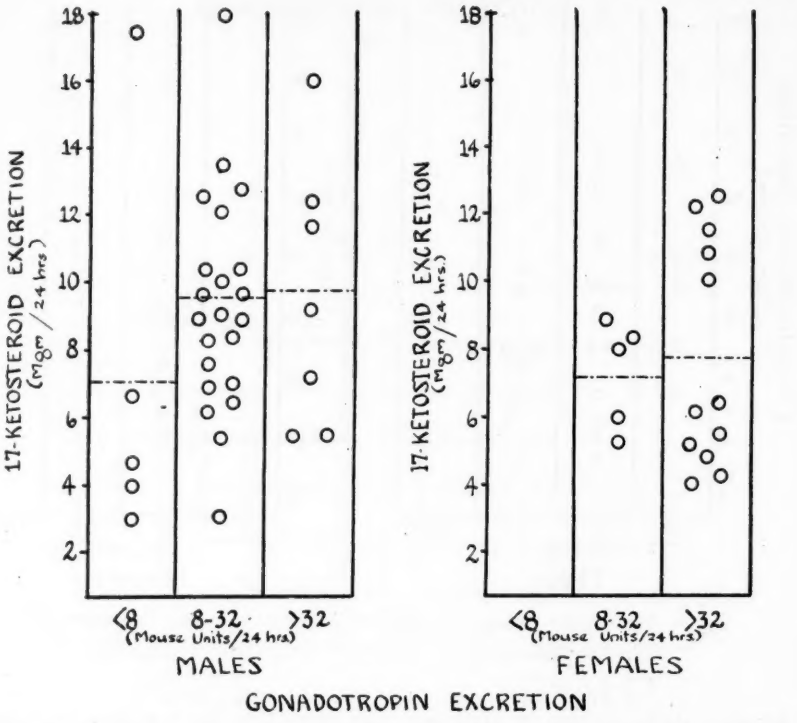

FIG. 5. *Relationship between excretion of 17-ketosteroids and gonadotropin in the well-aged.* The broken lines indicate the mean ketosteroid excretion levels in the groups with decreased, normal and increased gonadotropin titers for both sexes.

twenty-four hours, with a mean value of 0.33 (St. E. 0.06). Although the difference between the means of the two sexes in the aged group is of borderline statistical validity ($t = 2.3$, $p = .02$), the intragroup range is wide, and overlap between the sexes great. Thus it was our impression, on the basis of this small series, that cortin excretion was not significantly affected by either sex or the aging process. No correlation was demonstrable between cortin excretion and either gonadotropin or 17-ketosteroid excretion in either sex.

Osteoporosis

The degree of osteoporosis was rated as 0, 1+ (slight), 2+ (moderate), 3+ (severe), or 4+ (extreme). No vertebrae were considered to show extreme osteoporosis. The differences between the sexes and distribution of ratings may be seen in Figure 6. The mean for males is 1.6+; that for females, 2.4+. All the females showed more than a slight degree of change,

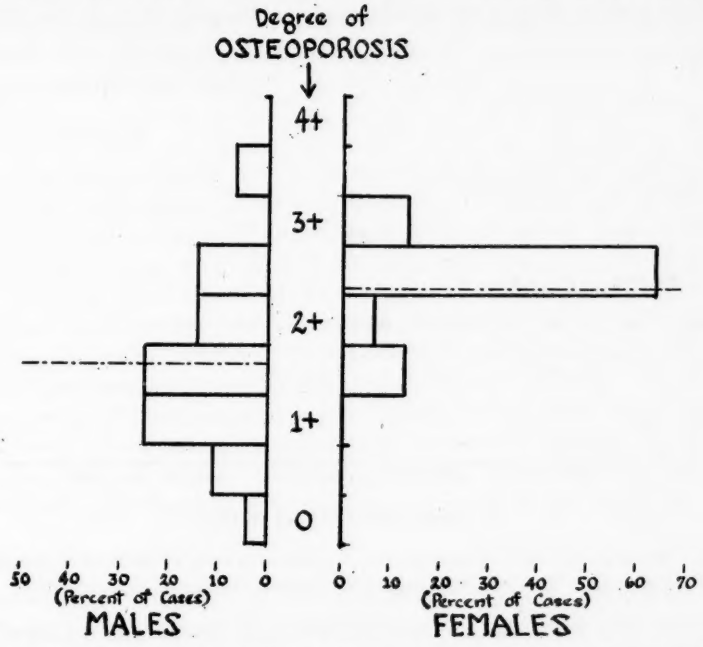

FIG. 6. *Distribution and variation of degree of osteoporosis in well-aged males and females.* The broken lines indicate the mean degree of osteoporosis in the two groups. Osteoporosis is rated from 0 to 4+, to represent zero, slight, moderate, severe and extreme osteoporosis respectively.

whereas in males 40 per cent were considered to have no more than slight osteoporosis. No correlation was noted between age and degree of osteoporosis for either sex, nor was there any relation between parity or duration of time since the menopause. Calcium intake as roughly estimated by dietary history did not bear any relationship to the severity of osteoporosis. If, in senility, osteoporosis is promoted by an increasing ratio of anti-anabolic corticosteroids to anabolic androgen, one might anticipate a correlation between the degree of bone dissolution and the ratio of keto-steroids to cortin as observed in individuals within the group. No such relationship could be established for either sex (Fig. 7), nor was there any

correlation between ketosteroid or cortin excretion alone and osteoporosis.

Vascular calcification

Calcification of the large arteries of the leg as revealed by x-ray films was rated from 0 to +++. The former indicated no visible calcification, whereas the latter was applied to those cases in which a considerable length of vessel could be clearly outlined. By this method there was a markedly greater degree of calcification in the men than in the women

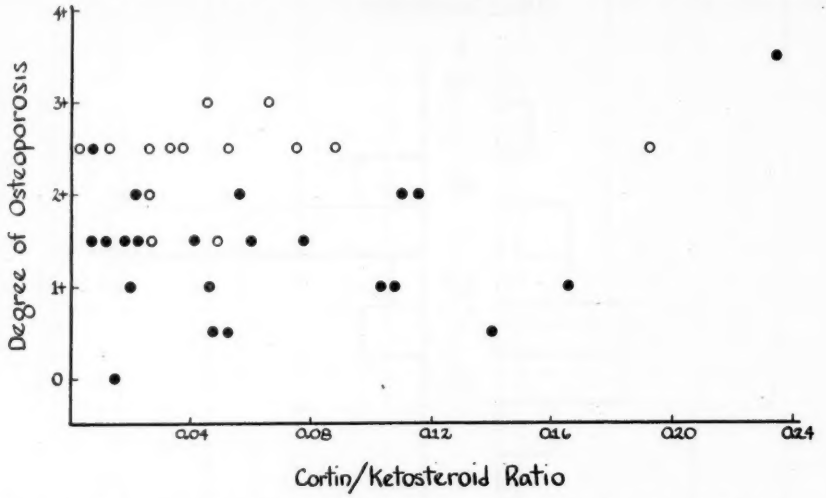

FIG. 7. *Variation in degree of osteoporosis in relation to ratio of cortin to 17-ketosteroid excretions.* The open dots represent females; the closed dots, males.

(mean of 1.6+ for males; 0.64+ for females). There was no correlation with age, no greater amount in the diabetics (all of whom were men), no correlation with clinical hypertension (systolic or diastolic), and no relationship to degree of vascular sclerosis estimated by palpation of accessible peripheral arteries.

Comparison of degree of vascular calcification with the various urinary hormone studies failed to disclose any correlation.

Climacteric

Of the group of women 7 (41 per cent) had had typical menopausal symptoms, but a similar number had had no symptoms at all. In the remaining 3 symptoms had been minimal. Those women with an asymptomatic menopause showed a gonadotropin excretion at the time of this study no different from that in women who had had fairly severe symptoms.

Of the men, only 1 (3 per cent) had had rather suggestive symptoms of a

climacteric, including hot flashes, nervousness, loss of potency and libido, and weakness. This had occurred at the age of 60 and a diagnosis of male climacteric had been made by his physician. At the time of our study he was 67 and had a 17-ketosteroid excretion of 16.0 mg. per twenty-four hours and gonadotropin excretion of 64 m.u. One other man aged 69 was experiencing some nervous and vasomotor symptoms at the time of our examination and had lost his potency and libido during the preceding year; the total symptomatology, however, was not quite so suggestive as in the first case. His 17-ketosteroid excretion was 9.8 mg. and gonadotropin, 16 m.u. per twenty-four hours.

Senile skin changes

An attempt was made to rate the degree of senile change in the skin by the criteria noted above. It was observed that within the group there was a distinctly higher degree of senile change with advancing age, and this was comparable in both sexes. Ketosteroid excretion was somewhat lower in those men with most advanced skin changes, but this was not of statistical significance and was not found in women. Similarly, there was a suggestive but not statistically significant correlation between the degree of osteoporosis and skin change in men, but this was not found in women.

The cortin: ketosteroid ratio was compared to the degree of skin change; but as with osteoporosis, no significant correlation could be found. The mean ratio for men with severe skin changes was higher than for those with minimal changes, but this difference was not statistically valid ($p = 0.3$).

Sexual potency and libido

Despite the obvious limitations of obtaining such data from men of this age group, an attempt to evaluate these sexual functions was made. The term "sexual potency" in this instance is used to mean the ability to obtain penile erections, either spontaneously or with stimulation. Of the 35 men, 16 claimed retention of this ability; 19 had lost it. Within this group there was no difference in mean age or age distribution between those who were potent and those who were not. Similarly, there was no difference in ketosteroid, cortin, or gonadotropin excretion. Of those who had lost their potency, the mean age of loss was 70.5 years. The mean age of last sexual intercourse of the entire group was 70.0 years, though in many cases this occurred at an early age due to death or separation from the spouse, and potency continued. In most instances loss of libido was experienced at approximately the same time as loss of erectile potency, though in a few cases libido persisted for many years after potency was lost. The mean ketosteroid, cortin, and gonadotropin excretion was the same for the group with persistent sex desire as for the group which had lost it.

Prostatic hypertrophy

The degree of benign prostatic hypertrophy was estimated by rectal examination, and enlargement was rated on a 1+ to 4+ scale. Those cases in which prostatectomy had previously been performed for obstruction were considered 4+. There was no demonstrable relationship between the degree of enlargement and the 17-ketosteroid excretion. There was, however, a suggestion of decreased gonadotropin excretion in those cases with the larger degrees of hypertrophy. Thus, those subjects considered to have no enlargement or only slight enlargement had a mean gonadotropin titer of 41.1 m.u., whereas those in the 3+ and 4+ categories had a mean level of 21.6 m.u. Statistically, this difference is not great enough to be significant ($p=0.1$); however, it may warrant further study with a larger group. There was no difference in gonadotropin excretion between those who had had prostatectomies previously and the rest of the 3+ and 4+ group.

Testicular size and consistency

Size and consistency of the testes was estimated and certain correlations attempted. The average testicular length was 3.3 cm., which is somewhat below the generally accepted range of 4.0 to 5.0. cm. As regards consistency, in all but 2 of the group (94 per cent) the softening was definite, and in 8 of these (23 per cent) the change was considered to be marked. No relationship could be established between either testicular size or consistency and ketosteroid excretion or potency. Gonadotropin excretion was similarly unrelated.

Axillary hair

An estimate of the amount of axillary hair present was made without an attempt to determine rate of growth after shaving. Despite possible individual variations, 11 men (32 per cent) had a very definite decrease, showing no hair at all or a very scant growth. Eleven others (32 per cent) had certainly less than the average amount, though conceivably within a normal range. The remainder (36 per cent) had a growth considered to be about average but several of these stated that this represented a decline from their young adult status. When these findings were compared with ketosteroid or cortin excretion, in neither case could any relationship be established. Axillary hair growth similarly was not related to the palpable state of the testis.

Estimations of facial hair growth could not be made satisfactorily in this group. Changes in pubic hair corresponded fairly well with those for axillary hair but decreases were much less marked and were difficult to measure.

Eyes

Careful examination of the eyes of 25 men of this group was made by a competent ophthalmologist, with special attention to those changes in the lens and cornea which are generally considered part of the aging process. Fifteen (60 per cent) had clear lenses, whereas the remainder (40 per cent) showed varying degrees of lenticular opacity. An attempt to classify the latter was not considered feasible. In the examination of the cornea, only 1 case of senile gutate keratitis was found (4 per cent). Hassall-Henle bodies were found in 13 (52 per cent), whereas the remainder (44 per cent) had clear corneae.

Attempts to correlate these changes with any of the hormonal excretions or other clinical findings were not revealing, except for an increasing incidence of cataracts with advancing age, within the group.

DISCUSSION

Much of the early work on gonadotropin excretion was performed without the advantage of a selective precipitation procedure or a very sensitive method of bioassay. Österreicher (6) was able to demonstrate activity in the urine of 11 of 53 men between the ages of 50 and 91, whereas he could detect none in the case of 33 young males. Saethre (7) encountered more positive results with urines in a group of elderly men than he did in a group of young men. Heller, Heller, and Sevringhaus (8), using a method sensitive enough to detect gonadotropin in all normal adult males, did not encounter higher values in 16 men ranging in age from 60 to 95 years than in 25 men between 20 and 60 years of age.

Our own findings indicate that a certain proportion of elderly men excrete higher amounts of gonadotropin than normal. High gonadotropin excretion, of course, suggests primary gonadal failure. That aged males show a much lower incidence of elevated gonadotropin values than do females, is in accord with the common belief that primary gonadal failure is less common in elderly men than in women. It is of interest, however, that the incidence of abnormally high values in men was as great as 20 per cent; and moreover, that of these 7 men, only 1 had at any time experienced symptoms suggestive of a climacteric state.

As to the low incidence of symptoms, two interpretations are plausible. It is possible that a decline in gonadal androgen secretion is so gradual that a tolerance develops concomitantly. A second possibility is that loss of tubular function alone, without failure of Leydig cell secretion is responsible for the elevated gonadotropin excretion. High values have been described in cases of simple azoospermia (9, 10, 11). In any event, an elevated gonadotropin output in older men, although fairly common, is not usually

accompanied by a climacteric syndrome. Heller, on the other hand, has reported that a group of men free of climacteric symptoms all excreted normal quantities of gonadotropin, whereas, without exception, a group with typical symptoms showed high levels of output (12). During the year's time while the present study was in progress an intensive search was made for patients having complaints consistent with the male climacteric. All outpatient departments of the hospital were canvassed for likely candidates. Only 2 were referred to the endocrine clinic and both of these proved to be suffering from purely psychiatric or functional disorders. Thus, again, it is evident that the incidence of abnormally high gonadotropin excretion in older men is considerably in excess of the frequency of symptoms which could be attributed to a male climacteric.

The values for gonadotropin excretion in the female group require comment. Of particular interest is the occurrence of normal values in 5 of the women. It is generally believed that a rise in gonadotropin excretion invariably accompanies primary gonadal failure. Heller and Heller found a sustained elevation in 3 women even beyond the age of 70 (13). The present findings are not in accord with the concept that high levels are invariably encountered during senility. It is easily conceivable, of course, that the range of values encountered immediately after the menopause overlaps the normal range, but with every individual still having experienced an absolute rise, *e.g.*, from a previous level of 8 units to a postmenopausal level of 32. The results, however, suggest that another element also may be involved. Four of the 5 women who showed normal values were 30 or more years past the menopause. Thus, it is possible that with advancing age a previously existing hyperactivity of the anterior pituitary may abate. Perhaps this represents a degenerative change rather than one of simple adaptation, because in the group of aged males, 5 had values which were actually lower than normal.

The observed decline in 17-ketosteroid excretion in aged subjects is in accord with the findings of others (14–19). The failure to demonstrate any difference between the mean values for the seventh, eighth, and ninth decade suggests that the senile decline has by this time reached its lowermost level. The relatively greater depression of 17-ketosteroids in aged males than in females has not previously been reported. In females the drop in 17-ketosteroid level to 60 per cent of that expected in young adults of the same sex is undoubtedly attributable to a decline in that component of adrenal cortical secretion which is composed of androgen-like precursors. With the dual source of 17-ketosteroids in men, the more extreme fall to 40 per cent of normal is susceptible to two possible interpretations. Either the adrenal secretion of precursors is more seriously impaired in men that in women or both adrenal and testicular function are concomitantly de-

pressed. The first alternative is not easy to accept. If within the group of men, the degree of 17-ketosteroid depression and gonadotropin elevation had been found to be correlated, one would suspect those individuals with low ketosteroids and high gonadotropin to have depressed Leydig cell function. However, an impairment of pituitary responsiveness, as already discussed, would prevent such a correlation from being manifest and the effect of tubular failure on gonadotropin output adds further to the confusion. Adrenal androgen secretion apparently has little if any effect on gonadotropin output, because in Addison's disease there is no elevation of gonadotropin excretion (9, 11). All things considered, it is reasonable to assume that the relatively severe decline in 17-ketosteroid excretion encountered in old men is accounted for by a combined partial diminution of both adrenal and testicular precursors. It must be kept in mind that this conclusion is based on statistical differences and that the variables affecting urinary assays are too complex to permit an evaluation of gonadal status in any given individual.

The failure of cortin excretion to diminish in late life along with 17-ketosteroids is of considerable interest. This is another instance of a dissociation between the output of cortin-like and androgen-like material from the adrenal gland. For example, it has been shown that during reactions to stress, 17-ketosteroid excretion may be depressed in spite of a sustained elevation in cortin excretion (4, 20). Such a disparity implies that 17-ketosteroids are formed, at least in part, from adrenal precursors other than the formaldehydogenic corticoids. It further denotes the presence of either a dual and selective responsiveness of the adrenal cortex to a single pituitary hormone, or points toward the existence of two separate pituitary hormones—one which promotes the secretion of androgenic material excreted as 17-ketosteroids and another which incites elaboration of cortical hormones of the formaldehydogenic group. Acceptance of the first alternative would mean that senility is accompanied by a selective impairment of the capacity of the adrenal to elaborate androgen. The second alternative leads to the conclusion that in senility a separate pituitary hormone concerned only with adrenal androgen secretion is produced in decreased amounts. In spite of the apparent physical homogeneity of existing purified extracts of adrenocorticotropic hormone, it is quite possible that two substances with different physiologic activities are present. This would account for the concomitant rise of both urinary 17-ketosteroids and corticoids after ACTH injection (21, 22).

The sustained secretion of corticoids in the presence of a decline of androgens may be of considerable physiologic importance during the senile age period. Albright (23) has proposed that such a consequence can, in part, account for the osteoporosis of late life. Loss of the anabolic support

probably given by adrenal androgens without a comparable loss of the antianabolic corticoids would provide a ratio between the two which might favor protein catabolism and hence lead to loss of bone matrix. Such an hypothesis might also be invoked to explain some of the atrophy which occurs in other tissues during the aging process. Albright has pointed out that in the female the loss of estrogen at the menopause impairs osteoblastic activity and also deprives the female of the small anabolic activity contributed by this hormone. At this age the male still secretes testicular androgen in good quantity. Later in life male and female hormones in both sexes are both low but total androgen production (as reflected by 17-ketosteroids) does not decline to as low an absolute level in senile men as it does in women.

It is of interest that no significant positive correlation could be established between any of the various gradations of senile change and the levels of hormone output among the subjects studied. If any relationships of this kind had been demonstrated, the case for an endocrine factor as a contributing cause of degenerative processes would be strengthened. It is possible that a larger sample of subjects showing a wider variation in senile characteristics might uncover such correlations. The evidence, however, is clear that senile men and women, as a group, show a depressed 17-ketosteroid output without a decline in cortin excretion and that the potential catabolic effect of such a shift in hormone balance might lead not only to osteoporosis but also to other degenerative alterations. That the administration of sex steroids, such as testosterone and estrogen, leads to a positive nitrogen and calcium balance in patients with senile osteoporosis has already been demonstrated (23). It has also been shown that estrogen, when applied to the skin of senile women, induces a return of the atrophic epithelium to a condition more nearly approaching that of normal (24, 25, 26). Further evidence bearing on the importance of the decline in sex hormones in relation to aging would be derived from the prolonged administration of hormones to a carefully followed group of subjects. For an ideal experiment, one should institute treatment at the age of 45 or 50 and continue it for many years. It is discouraging to contemplate the practical difficulties and pitfalls of such an undertaking.

SUMMARY

1. In a group of senile subjects comprising both sexes, 17-ketosteroid excretion was markedly lower than normal, whereas the output of cortin was unchanged. The excretion of 17-ketosteroids was no lower in the ninth decade than in the seventh. The ratio of cortin to 17-ketosteroids could not be correlated with the various degrees of osteoporosis or other senile changes among subjects within the group.

2. The depression of 17-ketosteroid output in senility was relatively greater in men than in women. This may be attributable to a decrease of both adrenal and testicular precursors in the former.

3. Gonadotropin output exceeded the normal range in 70 per cent of the aged women and in 21 per cent of the aged men. Women in the late postmenopausal period, however, showed a definite decline toward the normal range. There was no clear correlation of gonadotropin excretion with 17-ketosteroid excretion or with any of the clinical observations which were made on the subjects. Some of the senile men excreted abnormally low quantities of gonadotropin. Thus, in late life two opposite influences may act upon the pituitary—primary gonadal failure which tends to cause an increased output, and senile change which promotes a decreased output.

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THE DURATION OF ANTIDIURETIC ACTION OF PITRESSIN SOLUTION AND OF PITRESSIN TANNATE IN AQUEOUS AND OIL SUSPENSIONS*

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INTRODUCTION

Several investigations (1–7) have indicated that Pitressin Tannate, an insoluble complex of Pitressin[1] and tannic acid, possesses certain advantages over Pitressin and other water-soluble posterior lobe extracts for treatment of diabetes insipidus. The multiple daily injections of antidiuretic hormone necessary with aqueous extracts and attendant disagreeable side reactions encountered with such treatment are eliminated with Pitressin Tannate. Polyuria is controlled by intramuscular injection of Pitressin Tannate in oil at intervals as long as four days. Despite these advantages, communications from physicians and self-treated patients have revealed wide variation in required frequency of treatment in order to maintain satisfactory control over urine volume. These variations were not associated with changing status of the diabetes nor, as shown subsequently, with varying amounts of antidiuretic hormone in the preparations.

This communication presents experimental data in hydrated rats showing 1) the duration of antidiuretic action of Pitressin Tannate and Pitressin, 2) the effect of certain oily vehicles on the duration of antidiuresis from single injections of Pitressin Tannate, 3) a procedure for laboratory evaluation of the repository characteristics of Pitressin Tannate in Oil, and 4) a comparison of such data and the reported clinical response to three lots showing varied depot effects in patients with diabetes insipidus.

METHODS

Hormonal activity of Pitressin and Pitressin Tannate is expressed in pressor units, this being defined as the equivalent pressor activity of 0.5 mg. of U.S.P. Standard Posterior Pituitary Powder (8). To determine the pressor activity of Pitressin Tannate, the powder was suspended in glacial acetic acid which, on dilution to 50 per cent concentration with water, results in separation of the tannate complex and solution of Pitressin.

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[1] Parke, Davis and Company registered trademark for β-hypophamine.

Aqueous and oil suspensions of Pitressin Tannate were made by milling in a small steel ball mill with the suspension medium. Evaluation of the antidiuretic activity of Pitressin and the repository antidiuretic characteristics of Pitressin Tannate was based on their ability to cause a delay in excretion of orally administered water in rats. This was measured quantitatively by adaptations of the Burn technic (9).

Male Sprague-Dawley rats weighing 150–200 Gm. were maintained in constant-temperature quarters several days prior to use. Food cups were removed sixteen hours before hydration. Warm tap water was given by stomach tube in a volume equal to 5 per cent of body weight, following which the rats, in groups of four, were placed in screen-bottom cages over a glass funnel. Urine volume was recorded at 15-minute intervals, the antidiuretic effect of injected hormone being estimated by comparing the time of maximal excretion of ingested water in control groups with that in treated groups.

Pitressin and aqueous suspensions of Pitressin Tannate were injected subcutaneously; and oil suspensions, intramuscularly. Injections were given either immediately before hydration with water or, in the duration experiments, at some recorded interval prior to administration of water.

EXPERIMENTAL

Preliminary to investigations of the repository properties of Pitressin Tannate either in aqueous or oil vehicles, dose-response curves for an aqueous suspension of Pitressin Tannate and for a solution of Pitressin were prepared for comparison. In both experiments the antidiuretic hormone was injected immediately prior to hydration.

The two curves, plotted semi-logarithmically are shown in Figure 1. The antidiuretic response to Pitressin appears to be approximately linear as the dose was increased up to 8–12 milliunits. In amounts greater than this, there occurred no further delay in diuresis from water administration, a result which conforms closely to the observations of Heller and Urban (10) in similar studies. In the range of 9–36 milliunits, the dose-response curve for Pitressin Tannate, like that for Pitressin, was linear. However, the slope of the curve was much steeper and the duration of antidiuresis from the larger amounts of this hormone complex was much greater than that observed for Pitressin itself.

Another approach to the acquisition of comparative data on the duration of antidiuretic effects from administration of Pitressin and Pitressin Tannate was made by studying the extent of delayed diuresis in rats hydrated at various times *after* injection of the hormone preparation. In Table 1 are shown the experimental results with Pitressin solution. This solution was injected in varying doses at zero, one-half, one, two and three hours

prior to oral administration of water. Results in the first column of Table 1, when injection of Pitressin and hydration were simultaneous, conform to those shown in Figure 1. After one-half hour, however, the antidiuretic

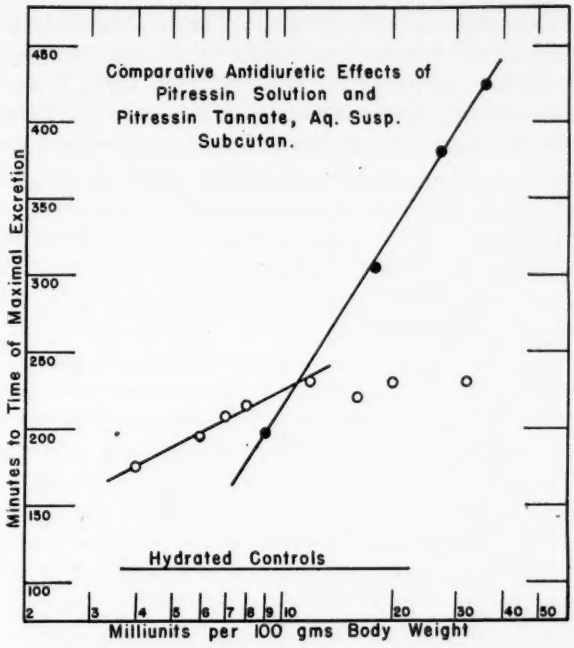

FIG. 1. Dose-response curves for Pitressin (open circles) and Pitressin Tannate, aqueous suspension (closed circles).

TABLE 1. DURATION OF ANTIDIURETIC EFFECTS OF PITRESSIN IN RATS

Dose of Pitressin (milliunits)	Time between injection of Pitressin and hydration				
	0 hr.	½ hr.	1 hr.	2 hrs.	3 hrs.
	Time in minutes to maximal excretion				
4*	164	138	118	92	77
8	204	161	141	114	—
16	208	179	—	111	83
32	216	180	—	110	87
Hydrated controls	108.6 ± 5.5**				

* At least 4 groups of 4 rats each were used for each value in the table.
** Standard error of the mean (32 groups).

effects of corresponding doses were definitely less than at zero time. After one hour, it would appear that an effective concentration of the hormone no longer exists in the body even at high dose levels.

With Pitressin Tannate, either in aqueous or oil medium, it could be anticipated from results already shown in Figure 1 that antidiuretic effects could be obtained over a much longer interval of time between administration of the hormone and hydration of the animal. Table 2 is a compilation of data from two groups of experiments, one in which the Pitressin Tannate was administered in aqueous suspension and another in which the same

TABLE 2. PITRESSIN TANNATE: AQUEOUS SUSPENSION VS. OIL SUSPENSION

Dose/100-Gm. rat		Time between injection and hydration (hrs.)	Aqueous suspension		Peanut-oil suspension	
milliunits	μg.		Number of groups*	Av. minutes to time of maximum excretion	Number of groups	Av. minutes to time of maximum excretion
9	2	0	9	214	7	289
18	4	0	9	279	5	361
27	6	0	4	365	3	419
36	8	0	4	431	3	++
90	20	0	1	++**	2	++
180	40	0	1	++	2	++
270	60	0	1	++	2	++
360	80	0	1	++	2	++
9	2	2	4	123	2	126
18	4	2	4	177	8	212
27	6	2	4	231	8	280
36	8	2	1	++	2	273
18	4	4	4	136	8	182
27	6	4	4	123	8	++
36	8	4	4	152	—	—
90	20	4	1	++	—	—
90	20	16	1	119	5	137
180	40	16	1	128	7	179
270	60	16	1	122	12	193
360	80	16	1	318	15	211

* Four rats per group.

Control rats—time of maximum excretion = 108.6 ± 5.5 (Std. error of the mean) (32 groups).

** More than seven hours.

lot of hormone was injected in peanut-oil suspension. The time intervals between injection and hydration of the rats were 0, 2, 4 and 16 hours and the dose ranges were 9 to 360 milliunits, depending in part on the interval between injection and water administration.

In those experiments in which hydration of the animal and injection of Pitressin Tannate were simultaneous, results with the aqueous suspension of the hormone agree well with the data shown in Figure 1 over the corresponding dose range. With larger amounts (90–360 milliunits) both aqueous and peanut-oil suspensions of Pitressin Tannate delayed diuresis for more than seven hours. Usually the accumulation of data was terminated at this time, since the quantitative character of the antidiuretic response to the larger doses of hormone is obscured somewhat by the prolonged anuria, lack of food, accumulated metabolic water and tendency toward water intoxication. These data are not recorded except, cryptically, as greater than seven hours. As the interval between administration of Pitressin Tannate and hydration of the rat was increased, larger doses of hormone were required to cause an antidiuretic effect, although even after sixteen hours results were indicative of continued presence of hormonal activity in the body, provided the amount injected initially was sufficiently great. The repository character of the response to Pitressin Tannate in peanut oil was more striking than with aqueous suspension and showed a graded response with increasing doses of this preparation. Results would indicate that 180 milliunits of the lot of tannate employed was required for an effective antidiuretic action sixteen hours after administration.

From observations on the enhancing effect of peanut oil on the duration of antidiuretic activity of Pitressin Tannate, it seemed desirable to study the same phenomenon using castor oil. This oil had been shown previously (unpublished) to possess unusual properties of potentiating and prolonging biologic responses to testosterone and to chorionic gonadotropin in single doses given subcutaneously or intramuscularly. Comparative results on the prolongation of antidiuresis with Pitressin Tannate suspended in peanut oil and in castor oil are shown in Table 3.

In these studies, the time between injection of the hormone suspension and the hydration of the rat was extended to periods up to sixty hours. This procedure had the dual tendency to exaggerate differences, as influenced by the suspension medium, in duration of effect from a given amount of injected hormone and to widen the differences in dosage required to induce threshold effects.

It is apparent from Table 3 that sixteen hours following injection of Pitressin Tannate, significant differences in the duration of antidiuretic action resulted from equal doses of the hormone in the two oils. The minimal dose of Pitressin Tannate in castor oil which provided physiologic

TABLE 3. DURATION OF ANTIDIURETIC ACTION OF PITRESSIN TANNATE* IN PEANUT OIL AND IN CASTOR OIL

Time between injection of Pitressin Tannate and hydration	Peanut oil				Castor oil			
	Dose of PT		Number of groups	Maximum excretion (minutes)	Dose of PT		Number of groups	Maximum excretion (minutes)
	μg.	milliunits			μg.	milliunits		
16 hrs.	10	30	4	122	5	15	14	170
	15	45	22	178				
	20	60	4	++	10	30	12	225
24 hrs.	20	60	4	127	10	30	8	170
	23	68	4	261	12.5	38	8	161
	25	75	4	288				
	30	90	4	++				
	40	120	4	++				
48 hrs.	30	90	7	114	15	45	4	109
	40	120	12	198	20	60	4	139
	60	180	16	189	30	90	8	111
					40	120	10	128
					50	150	4	120
					60	180	6	250
60 hrs.	100	300	4	124	100	300	4	154
	200	600	4	114	200	600	4	170
	325	975	3	271	325	975	4	++
	350	1056	4	246	350	1050	4	++
	400	1200	4	++	400	1200	4	++

* From 15 to 20 micrograms per 16-hour unit.

amounts of antidiuretic hormone in the body after sixteen hours was about 15 milliunits, whereas in peanut oil a considerably larger dose —45 milliunits—was required to exert a similar effect. Twenty-four hours following injection, the same general relationship between the two oils seemed to prevail, *i.e.*, approximately 30 milliunits in castor oil and 60–70 milliunits in peanut oil produced minimal antidiuretic action. Forty-eight and sixty hours following injection, the differences between castor oil and peanut oil tended to become less apparent. However, at these intervals, variability in experimental findings becomes so great as to obscure quantitative aspects of the problem.

The question next arose as to an explanation of the differences in

repository properties of commercial Pitressin Tannate in peanut oil as judged by communications from those using this preparation for treatment and control of diabetes insipidus. To study this problem, three lots were selected after careful appraisal of comments and criticisms directed toward them. These preparations are tabulated as "superior," when control of urine volume could be held at satisfactory levels by injections three to five days apart; "fair," when the polyuria could be controlled by injection every 48–72 hours; and "poor" (not released for general use), when preliminary clinical evaluation showed that daily injections were required.

Table 4 shows comparative experimental data on the three preparations.

TABLE 4. A COMPARISON OF 3 PEANUT-OIL SUSPENSIONS OF PITRESSIN TANNATE OF VARIED CLINICAL EVALUATION, SHOWING HORMONE CONCENTRATION AND ANTIDIURETIC ACTIVITIES AT SHORT AND LONG PERIODS AFTER INTRAMUSCULAR ADMINISTRATION IN RATS

Lot No.	Clinical comments	Solids per ml. of suspension	Aqueous extract of preparation		Minimal dose in oil (zero hour antidiuretic effect—70 minutes)		Minimal dose in oil (zero hour antidiuretic effect—120 minutes)		Minimal dose in oil (for 16-hour antidiuretic effect)	
			Antidiuretic assay—(Burn)	Pressor assay (Rowe)						
		mg.	U.S.P. units per ml.*	U.S.P. units per ml.*	μg.	milli-units	μg.	milli-units	μg.	milli-units
H965G	"Superior"	1.8	7.9	5.5	0.20	0.9	0.30	1.3	4	17
H1072J	"Fair"	1.7	6.8	5.0	0.38	1.5	0.57	2.3	31	120
F704J	"Poor"	1.0	5.8	5.5	0.45	2.6	0.90	5.2	54	311

* Original oil suspension.
Not less than 4 groups of 4 rats each were used for determination of above antidiuretic effects.

The lot number, the concentration of extract and "clinical" evaluation are given in the first three columns. Column 4 contains parallel antidiuretic (Burn method) and pressor assays of an aqueous extract of the three suspensions of tannate after removal of the peanut oil with ether and separation of the tannic acid complex by treatment with glacial and 50 per cent acetic acid. The results are expressed as U.S.P. units per milliliter of the oil suspension of Pitressin Tannate. Column 5 shows the dose of Pitressin Tannate in Oil which when injected simultaneously with water hydration caused a delay of seventy minutes from the control time of maximal excretion. In column 6 are the results of similar experiments in which the dose required to exert an antidiuretic effect equivalent to a 120-minute delay in water excretion was determined. Finally, in column 7 is given the dose of extract in oil suspension which when administered sixteen hours prior to the standard procedure of hydration of the rat gave an antidiuretic effect of magnitude sufficient to cause delay of water excretion by ninety minutes, *i.e.*, a maximal excretion time of two hundred minutes. In columns

5, 6 and 7 the dosage of extract given in micrograms is also expressed in milliunits calculated from the antidiuretic assay of column 4.

Examination of the data in column 4 indicates that the actual amount of antidiuretic (or pressor) hormone in the three lots of extract was essentially the same. Although it would appear by the Burn (6) method that there was less active substance in the "poor" preparation compared to the "fair" and "superior" extracts, differences were not revealed by the pressor method in dogs. Values given in columns 5 and 6 varied significantly among the three hormone extracts. These values represent respectively, dosages of Pitressin Tannate which yield at the site of injection an immediately available amount of Pitressin to induce minimal degrees of antidiuresis, *i.e.*, 70-minute and 120-minute delays in water excretion after uniform hydration. The variations ranged from amounts equivalent to 0.9 milliunits (70-min.) and 1.3 milliunits (120-min.) to 2.6 and 5.2 milliunits respectively, between the "superior" and "poor" preparations. Between lots H965G, H1072J and F704J the dosage ratios were in the order of 1, 2 and 4, showing immediately the better qualitative character of H965G than of the other two preparations.

These results were exaggerated in the experiments in which the time between injection of the extract and the antidiuretic test was extended to sixteen hours (Table 5, column 7). Here the corresponding doses of the "superior," "fair" and "poor" extracts were 17, 120 and 311 milliunits respectively—dose ratios of approximately 1, 7 and 18. To provide sufficient hormone for a detectable antidiuretic effect sixteen hours after administration, far more of F704J must be injected than of H965G. Once again, the "fair" lot lay intermediate between the other two.

DISCUSSION

The duration of effectiveness of aqueous extracts of the posterior pituitary is limited essentially by the time they remain at the site of injection and continue to be absorbed. That this time persists for no more than a few hours is evident from the data just presented, from studies by Heller and Urban (10) and Jones and Schlapp (11) on blood concentrations of antidiuretic, pressor, and oxytocic hormones in rabbits and cats, and from clinical experience with the frequency with which aqueous extracts must be administered in order to induce continuous effects. For such extracts, the factors of absorption, inactivation and excretion are operative within short periods of time. With Pitressin Tannate, no such limitation of duration of activity is encountered. Depending on the amount of hormone injected in tannate form, aqueous suspensions continue to release hormone into the body for long periods. This depot action is enhanced materially by use of oily vehicles, castor oil being somewhat superior to peanut oil in this regard.

Variation in the depot action of Pitressin Tannate in patients with diabetes insipidus has been recognized hitherto only from sundry reports of required frequency of dosage. This variation correlates well with dosages of corresponding extracts required for maintenance of the more precisely measurable state of antidiuresis in the hydrated rat. An extract considered "superior" because of its long-lasting action in control of polyuria in patients, was found antidiuretic in rats in far smaller doses than another preparation considered "poor" for clinical use. A lot of Pitressin Tannate having "moderate" depot action, therapeutically, occupied an intermediate position in the tabulated rat data. Such differences in repository characteristics are dependent on the firmness of the Pitressin-tannic acid binding rather than on the actual antidiuretic hormone content. This conclusion is based on differences between the "poor," "fair" and "superior" preparations, comparing the doses which induce minimal antidiuretic effects shortly after injection with the corresponding doses required for maintenance of an antidiuretic state for prolonged periods. Correlated with this reasoning is the fact that once Pitressin is released from its tannate binding, its effective life is short.

In the case of the "superior" lot H965G, an injection of 0.9 milliunit into a simultaneously hydrated rat delayed diuresis by seventy minutes. A dose of 17 milliunits of this same lot was required to induce essentially the same antidiuretic effect (delayed water-excretion time of seventy minutes) if administered sixteen hours prior to hydration. One may assume that of the 17 milliunits, 0.9 milliunit was being absorbed at the sixteenth hour—a dose ratio of nearly 19 to 1. In contrast, the "poor" lot F704J, which required 2.6 milliunits for minimal antidiuretic action at zero hour, had to be given in the large dose of 311 milliunits to obtain a similar effect at sixteen hours—a dose ratio of 119 to one. In the latter preparation it is evident that, due to the more unstable tannate binding, a much larger proportion of this extract was dissipated in the period of sixteen hours than with the more stable H965G. To evaluate Pitressin Tannate adequately for its ultimate efficacy in diabetes insipidus, it would appear necessary to determine not only the content of antidiuretic hormone but the repository characteristics as well.

SUMMARY

Employing a modified Burn procedure, the duration of antidiuretic action of Pitressin (a water-soluble posterior pituitary extract) and Pitressin Tannate (an insoluble complex of Pitressin and tannic acid) have been compared.

Unlike Pitressin, the action of which persists for only an hour or two even in larger doses, Pitressin Tannate administered as an aqueous suspension was effective for periods up to sixteen hours. Use of peanut oil or castor

oil as a suspension vehicle greatly prolonged the antidiuretic effects of Pitressin Tannate, castor oil being somewhat more effective in this regard.

Three lots of Pitressin Tannate described as "superior," "fair" and "poor" on the basis of frequency of injection required for control of diabetes insipidus in patients, were selected for study in the rat. Good correlation between the dose required for minimal antidiuretic action sixteen hours after injection and the clinical designation was obtained, despite evidence for equal concentration of antidiuretic hormone in the three preparations.

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THE EFFECTS OF *p*-HYDROXYPROPIOPHENONE ON THE THYROID GLAND OF EXPERIMENTAL ANIMALS*

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RECENT reports from France (1) indicate that *p*-hydroxypropiophenone shows a marked ability to alleviate the signs and symptoms of thyrotoxicosis. Perrault has postulated that the beneficial effects of this drug are due to an inhibition of the production of the thyroid-stimulating hormone. Although confirmatory evidence of this observation is lacking, the results and conclusions which have been reported make a careful investigation of the physiologic action of this compound essential.

In the course of our studies on the growth and function of the thyroid gland we have investigated the effect of *p*-hydroxypropiophenone on thyroid function in experimental animals. This report is the result of these studies.

MATERIALS AND METHODS

One hundred and twenty mature male rats of the Sprague-Dawley strain were used. In addition, 40 three-day-old chicks[1] were also used.

The experiments were set up in the following manner:

Experiment I. Four groups (10 animals per group) of male rats were given a low-iodine diet (2). *p*-Hydroxypropiophenone was injected as a saline suspension subcutaneously into three groups of these animals, once a day for ten days. Group 1 received 5 mg. daily, Group 2 received 50 mg. daily and Group 3 received 100 mg. per day. Group 4 served as untreated controls.

Experiment II. Four groups of male rats (10 animals per group) were given regular laboratory food and 0.1 per cent thiouracil in their drinking water. Group 1 received 5 mg. of *p*-hydroxypropiophenone daily. Group 2 received 50 mg. daily and Group 3 received 100 mg. daily. Group 4 served as uninjected controls. Injections of *p*-hydroxypropiophenone were made

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[1] Chicks were sex-linked males obtained from Harco Orchards, Easton, Mass.

subcutaneously once a day for fifteen days. Thiouracil was continued to the time of autopsy.

Experiment III. Four groups of male rats (10 animals per group) were given a low-iodine diet. Group 1 served as controls on this diet. Group 2 received *p*-hydroxypropiophenone mixed in the diet so that the daily intake of the drug was approximately 200 mg. per day. Group 3 received the low-iodine diet and 0.1 per cent thiouracil in the drinking water. Group 4 was given the thiouracil in drinking water and *p*-hydroxypropiophenone in the diet so that the intake was 200 mg. daily. Thiouracil was withdrawn from animals in this experiment forty-eight hours before autopsy. The *p*-hydroxypropiophenone was given for ten days.

Experiment IV. Forty three-day-old baby chicks were given a low-iodine diet. Group 1 received 0.1 per cent thiouracil in the drinking water. Group 2 received thiouracil and 100 mg. of *p*-hydroxypropiophenone per animal daily in the diet. Group 3 served as controls. Group 4 received the diet containing 100 mg. of *p*-hydroxypropiophenone per animal daily. *p*-Hydroxypropiophenone was continued for ten days. Thiouracil was withdrawn forty-eight hours before autopsy.

Twenty-four hours before autopsy all animals were given 1 microcurie of carrier-free I^{131} intraperitoneally. At autopsy animals were given sodium pentobarbital and the organs were removed, dissected free of fat and connective tissue and weighed on a Roller-Smith balance. They were then placed in 10-cc. vials containing 5 cc. of Bouin's solution. Counting of I^{131} was done in a Texas Company gamma-ray Geiger-Müller tube. The I^{131} in the thyroid tissue is expressed as a percentage of the injected solution, an aliquot of which was measured at the same time and under the same conditions as the glands.

The data are presented in Table 1.

EXPERIMENTAL RESULTS

The results obtained from rats injected with various amounts of *p*-hydroxypropiophenone[2] (Experiment I) are shown in Table 1. The data indicate that this compound in high doses produces a slight decrease in thyroid weight. However, the thyroid-body weight ratios are essentially unchanged. Radioactive iodine collection by the thyroid glands was depressed roughly in proportion to the amount of *p*-hydroxypropiophenone the animals received. This decrease was less apparent when the I^{131} collection was calculated per milligram of thyroid.

Adrenal weights of these animals indicated that *p*-hydroxypropiophenone

[2] *p*-Hydroxypropiophenone obtained from A. D. Mackay, 198 Broadway, New York, N. Y.

produced hypertrophy of these glands. All groups of animals receiving the drug had larger adrenals than did the controls. Pituitary gland weights of animals in this experiment were determined and no significant difference was observed in any of the groups.

TABLE 1

Group	*p*-Hydroxy-propio-phenone, mg./day	Thiou-racil	Body weight (grams)		Thyroid weight (mg.)	Thyroid wt. / body wt. ×100	Per cent I^{131} collected / thyroid gland	Per cent I^{131} (mg.)	Weight (mg.)
			at start	at autopsy					
				Experiment I (rats)					Adrenals
1	5	None	195	175	10.1	5.9	30.26	2.97	37.2
2	50	None	193	167	9.7*	5.8	25.59*	2.79	34.1
3	100	None	195	159	9.5*	5.9	21.01*	2.18	38.5
4	Controls	None	195	187	11.9	6.4	40.00	3.38	29.6
				*Experiment II*** (rats)					
1	5	Given	163	188	21.6	11.6	1.98	0.09	28.5
2	50	Given	162	159	19.3	11.8	2.06	0.13	37.4
3	100	Given	163	171	22.8	13.6	0.69	0.03	34.5
4	Controls	Given	162	198	25.6	12.9	0.67	0.03	30.6
				*Experiment III**** (rats)					
1	Controls	None	224	213	15.5	7.2	31.96	2.05	40.3
2	200 mg.	None	225	203	13.5	6.7	26.81	2.00	40.9
3	Controls	Given	224	229	29.6	12.9	34.21	1.16	34.1
4	200 mg.	Given	224	212	25.8	11.5	33.26	1.48	32.2
				*Experiment IV**** (chicks)					Testes
1	Controls	Given	—	58	6.3	10.6	9.29	1.23	13.5
2	100 mg.	Given	—	62	7.9	13.1	8.96	1.29	12.2
3	Controls	None	—	55	3.3	6.1	9.46	3.27	15.2
4	100 mg.	None	—	56	3.3	5.8	14.88	4.80	14.3

* $p = <0.01$, calculated by the method of Fisher (3).
** Thiouracil continued to time of autopsy.
*** Thiouracil withdrawn forty-eight hours before autopsy.

In Experiment II, in which the effect of *p*-hydroxypropiophenone on the induction of a thiouracil goiter was studied, no definite indication of goiter inhibition was obtained. However, animals receiving 50 mg. daily of *p*-hydroxypropiophenone showed an average thyroid weight of 19.3 mg., whereas the controls showed an average thyroid weight of 25.6 mg. Due to variation in thyroid weight within this experimental group, this difference was not statistically significant. Moreover, inspection of Table 1 shows that the thyroid-body weight ratios between these groups were essentially the same.

Since these animals received thiouracil until the time of autopsy, the uptake of I^{131} was small. There is no indication from these data that *p*-hydroxypropiophenone influenced the collection of radioactive iodine.

Adrenal weights of the animals receiving 50 and 100 mg. daily of *p*-

hydroxypropiophenone were increased. Pituitary weights were not significantly changed from control values.

In Experiment III the results suggested that the rats receiving the low-iodine diet or receiving thiouracil had slightly smaller thyroids when *p*-hydroxypropiophenone was given by mouth. However, these differences were not statistically significant; neither were the thyroid-body weight ratios for these groups. These results were similar to those obtained in Experiment I. The studies with I^{131} again indicated that the administration of *p*-hydroxypropiophenone decreased iodine collection in the thyroid. This was less apparent in the animals which received thiouracil, despite the fact that the antithyroid drug was withdrawn forty-eight hours before autopsy.

Adrenal weights of the experimental animals in this group were essentially the same as those of control animals.

In Experiment IV *p*-hydroxypropiophenone given to chicks did not result in any statistically significant difference in thyroid weights, although the averages might suggest that the compound increased slightly the size of the glands in birds receiving thiouracil. I^{131} studies with the non-thiouracil treated chicks indicated that *p*-hydroxypropiophenone increased slightly the collection of iodine. These differences, however, were within the normal variation found in chicks and were not significant.

The testes of these chicks were weighed and no difference was observed between experimental and control animals.

DISCUSSION

These studies show that *p*-hydroxypropiophenone in large doses has little influence on thyroid function as measured by I^{131} collection in the rat and chick. Moreover, no evidence of stimulation or inhibition of adrenal or gonadal function was observed.

The fact that this drug decreased I^{131} uptake by the thyroids of rats on a low-iodine diet might be significant from a physiologic standpoint. Since *p*-hydroxypropiophenone, however, is essentially half of the diethylstilbestrol molecule, the possibility of estrogenic activity being present in the compound has to be considered. We have tested our *p*-hydroxypropiophenone on uterine weight changes in immature rats and have found it to show estrogenic activity. No attempt has been made to measure this activity quantitatively. We have previously shown (4) that diethylstilbestrol and other estrogenic compounds will influence the collection of I^{131} by the thyroid gland. It is possible, therefore, that the effect of *p*-hydroxypropiophenone on the thyroid collection of I^{131} is due to its estrogenic activity. Since the adrenals of the rats were enlarged, another possible explanation is that the response of the thyroid was indirect and produced through secretion of adrenal steroids.

We have been unable to confirm the report of Perrault (1) that the development of goiter as a result of thiouracil administration is prevented by *p*-hydroxypropiophenone. Our experiments indicate that whether given orally or parentally, it fails to change the response of the thyroid to thiouracil treatment. Thus we do not feel that *p*-hydroxypropiophenone shows evidence of selective depressor action on the pituitary secretion of TSH.

Perrault's conclusion that the compound does not produce inhibition of ACTH secretion has been confirmed by our studies. In addition, his statement that *p*-hydroxypropiophenone does not interfere with pregnancy has also been confirmed. We have given the drug to pregnant rats without any obvious signs of complications.

From our studies, it would appear the *p*-hydroxypropiophenone shows no specific ability to interfere with the pituitary-thyroid relationship of the rat or chick. Its value as a therapeutic agent in clinical conditions, however, might require further consideration.

SUMMARY

p-Hydroxypropiophenone has been administered to rats and baby chicks. Results indicate that oral or parental administration to rats on a low-iodine diet decreases the collection of I^{131} by the thyroid. The compound failed to inhibit the formation of a goiter in animals treated with thiouracil. It is concluded that the drug is not a specific inhibitor of pituitary hormones.

Addendum

Since this article was submitted for publication, a report has appeared by R. Guillemin (*Presse méd.* **59:** 799 (June 9) 1951) which indicates that the prolonged administration of large doses of *p*-hydroxypropiophenone causes no inhibition of the production of gonadotropins, thyrotropin or somatotropin by the pituitary.

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THERAPEUTIC STUDIES IN HYPERTHYROIDISM: SODIUM 5-IODO-2-THIOURACIL*

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IT HAS been previously suggested (1) that the ideal treatment of hyperthyroidism will result in a restoration of the thyroid to normal physiologic relationships and normal histologic structure. The fact that subtotal thyroidectomy seems to accomplish this in some cases may be explained by supposing that the reaction to surgery induces an inhibiting mechanism. This theory of an inhibiting mechanism receives support from the reported effect of the adrenal steroids on thyroid function (2). The antithyroid thiouracil drugs, however, do not result in restoration of thyroid parenchyma to normal (3).

The synthetic combination of iodine and thiouracil[1] (Barrett, *et al.* (4)) raised the possibility that involution to normal histologic structure, prevention of goitrogenicity, and induction of normal physiologic relationship might be accomplished.

The advantages of sodium 5-iodo-2-thiouracil (hereafter called iodo-thiouracil) are based on the following theoretical considerations: 1. The hyperthyroid gland has a great affinity for iodine; therefore, an effective and selective concentration of iodinated compounds in the thyroid might result. The dosage required could well be smaller than that usually employed with noniodinated thiouracils and the control of thyrotoxicosis more rapid than with the use of noniodinated thiouracils. If the assumption is made that iodo-thiouracil will yield free iodine in the gland, in view of the ability of free iodine to inhibit thyrotropin (5, 6, 7), the hyperplasia, hyperemia and friability of the thyroid should be diminished. 2. If smaller doses of this iodinated compound are required for the control of thyrotoxicosis than those usually employed with noniodinated thiouracils, the danger of agranulocytosis, leukopenia, and other drug reactions may be diminished or avoided. Thus an effective antithyroid compound might be

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[1] Sodium 5-iodo-2-thiouracil. The iodine and the thiouracil each accounts for about half the molecular weight. We are very grateful to Ciba Pharmaceutical Products, Inc. for the generous supply of this drug.

found which is not goitrogenic and only slightly toxic. This theoretical elaboration has been tested as summarized below:

F. X. Gassner *et al.* (8) and The Ciba Research Laboratories attempted to clarify and to differentiate between the actions of iodo-thiouracil, of thiouracil and of propyl-thiouracil, alone, and with admixture of iodide. Essentially two criteria were employed for this evaluation:

A. *Degree of antithyroid action*

I. *Changes in oxygen consumption rate.* Barrett and Gassner (9) found that suppression of oxygen consumption in rats treated with thiouracil, thiouracil+iodide and iodo-thiouracil was of the same degree (−20 per cent), but the rebound to normal consumption occurred in six days (average) after thiouracil and thiouracil+iodide, whereas it required eighteen days after iodo-thiouracil.

II. *Radioactive iodine uptake.* Money (10) in chicks and Williams, *et al.* (11) in rats, found that the uptake of radioactive iodine was blocked more effectively with iodo-thiouracil than with thiouracil alone. This blocking effect was slightly greater in rats which were given potassium iodide than in those which were given iodo-thiouracil, although these compounds contain the same amounts of iodide.

III. *Inhibition of circulating TSH.* Using chicks, Gassner *et al.* (8) found that iodo-thiouracil, when given with TSH, did not produce the augmentation in thyroid weight observed with thiouracil or propyl-thiouracil, with or without added potassium iodide. The probable inhibition of TSH by the *in vitro* addition of iodo-thiouracil was checked, and it was observed that TSH potency was reduced by iodo-thiouracil, was augmented by propyl-thiouracil, and was also augmented by thiouracil alone, but to a lesser degree.

IV. *Thyroid iodine content.* In studies conducted on rats and chicks, Barrett *et al.* (12) and Gassner *et al.* (8) found that the loss of thyroid iodine in the group fed with iodo-thiouracil was 82 per cent of that in the controls. With thiouracil and propyl-thiouracil, the loss was about 95 per cent and only slightly less when potassium iodide was given simultaneously.

B. *Degree of goitrogenic action*

I. *Changes in thyroid weight.* Barrett *et al.* (12) found that iodo-thiouracil produced an increase of only 50 per cent above the control thyroid weight; thiouracil, about four times the control weight; and propyl-thiouracil, about twice the control weight. When potassium iodide was added to these compounds, the response was reduced to one and one-half times the control weight.

II. *Changes in histologic structure* (12). The iodo-thiouracil glands were

pale, firm and small. The microscopic picture resembled that of normal glands with somewhat smaller follicles. There was a good quality of colloid and vascularity was about normal. The thiouracil and propyl-thiouracil glands were large, soft, hyperemic and greatly hyperplastic, with the colloid reduced to a minimum and vascularity greatly increased. When potassium iodide was added to these compounds, the thyroids were large, moderately firm and hyperemic. The microscopic picture showed some areas with large follicles and flattened epithelial cells, whereas other areas showed marked epithelial hyperplasia, intrafollicular hemorrhage and increased vascularity.

III. *Influence of iodo-thiouracil on the effect of exogenous TSH.*

The results are described under Part III of antithyroid action.

The experiments just discussed suggest that iodo-thiouracil is a potent antithyroid agent and possesses little or no goitrogenicity. Its behavior is not reproduced by the equivalent mixture of thiouracil and potassium iodide.

CLINICAL EXPERIENCE

Williams *et al.* (11) in 1949, reported the effects on 29 patients who received 40 courses of therapy with iodo-thiouracil, 5 of whom underwent surgery. Control of thyrotoxicosis was accomplished much more rapidly than with thiouracil. The maximal clinical response, as measured by decrease of the basal metabolic rate, occurred within ten days in 15 patients and within fifteen days in 21 patients. The patients who had not become euthyroid within fifteen days did not attain this state with continuation of therapy. The 5 patients who underwent surgery experienced a smooth operative and postoperative course. The glands were relatively avascular, non-friable, and revealed a good degree of involution. The dose used varied from 60 to 300 mg. of iodo-thiouracil given daily in equally divided doses at approximately eight-hour intervals. The usual dose was 150 mg. per day. The effects of 100 to 150 mg. daily were essentially as good as those of 200 to 300 mg daily. Exacerbation of thyrotoxicosis occurred upon changing from iodo-thiouracil to propyl-thiouracil, even though the latter in the same dosage had previously been effective.

McClintock (13) reported that the thyroid glands of patients prepared for surgery with iodo-thiouracil were less vascular and more fibrous than the glands treated by other methods. The dose he used varied from 100 to 800 mg. daily, the greater dosage being used in the severe cases of thyrotoxicosis. The average length of treatment was from two to three months. The author is of the opinion that the dosage of 300 to 400 mg. for three to four months is more satisfactory.

METHODS AND MATERIALS

Twenty-six unselected patients with thyrotoxicosis were given 30 courses of therapy with iodo-thiouracil. Eleven patients were hospitalized during the complete course of therapy. Ten patients were hospitalized until the maximal clinical response was obtained and were subsequently treated as ambulatory. Five patients were treated as ambulatory from the first. Of the 26 patients, 23 were female and 3 were male; 8 were between the ages of 20 and 30, 5 between 30 and 40, 10 between 40 and 50, and 3 were above 50 years of age. All but one had diffuse thyroid enlargement and that one had a nodular toxic goiter. Of the 30 courses of therapy—in 14 the daily dose was 200 mg.; in 3, 200–400 mg.; in 2, 400 mg.; in 6, 600 mg.; in 1, 600–800 mg.; in 1, 200–400–600–800 mg.; in 1, 200–100–50 mg.; in 1, 400–200–100 mg., and in 1, 600–100 mg.

Emphasis was placed on obtaining several BMR determinations on each patient as control data before starting therapy. During the first two weeks of therapy, BMR determinations were made twice a week and thereafter at weekly intervals. Determinations of protein-bound iodine in the plasma (by the method of Chaney, (14)) were obtained before therapy commenced. Follow-up determinations of the protein-bound iodine were rendered unsatisfactory by the high iodine content of iodo-thiouracil. Tracer uptake studies with radioactive iodine were performed before treatment in all cases, and during treatment also in 4 patients. A tracer dose of 100 microcuries of radioactive iodine was given twenty-four hours prior to the count, which was made directly over the thyroid region. White blood cell counts were performed before treatment as a control, twice a week during the first two weeks of therapy, and at weekly intervals thereafter. The glands removed by surgery were weighed before blocks were taken for histologic studies. The remainders were subjected to chemical analysis for iodine content, following the method of Chaney (14) modified for tissues.[2] The histologic sections studied corresponded to two, three or four different

[2] Method for determination of total iodine and protein-bound iodine in glands:—A Waring blender was used to homogenize the weighed tissue, to which had been added 150 ml. of saline. Further attempts to homogenize the fibrous material, with a Potter homogenizer and a piston pump homogenizer, failed. The unhomogenized residue was centrifuged and washed four times; the washings were combined with the homogenized portion. The small quantity of remaining material (non-homogenizable) was not included in the analysis. The homogenate was diluted with saline so as to contain about 100–250 micrograms per cent of iodide, the total volume was measured and 3-cc. aliquots were taken for the actual analysis. From this point, the determination of total iodine and protein-bound iodine was carried out as in the Chaney method for protein-bound iodine in plasma (14), except that the digest was diluted with double-distilled water and an aliquot taken, in order that the final sample would fall within 4–10 micrograms per cent.

TABLE 1

Patient	Age	Sex	Dose of iodo-thiouracil	Initial		Maximal clinical response		
				BMR %	Pulse rate per min.	BMR %	Pulse	Days required
			(mg.)					
F.G.	20	M	200	+51	100	+ 6	72	7
G.G.	24	F	200	+46	112	− 3	88	7
K.A.	36	F	600	+53	132	− 4	88	7
K.M.	48	F	200	+89	fibrillating, more than 140	−20	80	7
H.E.	74	F	200	+24	fibrillating, more than 140	−12	fibrillating 72	7
B.D.	43	F	200	+36	92	+ 6	68	8
M.P.	29	F	600	+38	100	− 3	72	8
G.E.	48	F	400	+31	fibrillating, more than 140	− 3	64	8
P.V.	46	F	200	—	112	−14	60	9
E.O.	54	F	200	+42	88	−11	64	10
B.M.A.	49	F	200	+28	100	+ 6	80	11
C.H.	35	F	200	+37	88	+ 4	76	11
G.L.	39	F	600	+25	112		82	15
T.D.	39	F	200*	+23	92	+ 1	84	4
S.M.	40	F	200*		126		92	15
T.M.	53	F	200*	+34	100	+ 6	78	4
W.G.	46	F	600	+53	100	+14	78	16
S.V.	44	F	200	+58	108	+14	80	18
H.C.H.	39	M	600	+54	116	+ 4	72	19
G.B.	50	F	400	+23	96	−18	86	19
M.O.	42	F	200	+31	112	+ 8	84	21
W.J.	30	F	600	+59	112	+10	105	22
						Response slow and/or incomplete		
								Days under treatment
O.R.	20	F	200–800	+54	124	+19	116	90
C.S.	28	M	600–800	+47	112	+ 6	106	30
B.I.	24	F	200	+46	116	+19		29
G.M.	26	F	200–400	+32	104	+27	100	68

* As an adjunct to radioiodine therapy.

blocks of the same gland; and microhistometric determinations to obtain the mean acinar cell height were made, following the method of Starr (15). Of the 26 patients, 21 received treatment as a preparation for surgery, 2 as long-term therapy, and 3 as an adjunct to radioiodine therapy.

BIOCHEMICAL AND PATHOLOGIC RESULTS

To evaluate the results of the potency of this drug, the same criteria used in the experimental work on animals by Gassner, were taken into consideration, namely: (a) The degree of antithyroid action as measured by the maximal clinical response, blocking of radioiodine uptake, and thyroid iodine content. (b) The degree of goitrogenic action as measured by changes in thyroid size during treatment, by clinical observation, and by histologic changes, especially the mean acinar cell height.

Degree of antithyroid action

I. *Maximal clinical response* was measured by the decrease in the basal metabolic rate and pulse rate, the gain in weight and the disappearance of the symptoms of hyperthyroidism. As shown in Table 1, 10 patients had maximal clinical response within eight days, 7 within sixteen days, 5 within twenty-two days, and 4 responded slowly and/or incompletely. In each of the first three groups, the dosage varied from 200 to 600 mg. and in the last group from 200 to 800 mg. This corroborates the findings of Williams *et al.* (11) that the effects of the smaller doses are essentially as good as those of the higher doses. It was observed that when the smaller doses (such as 200 mg.) failed to control the disease, higher doses failed likewise, as seen in the 4 cases which responded slowly and/or incompletely.

II. *Radioiodine uptake.* A definite inhibition of the uptake of radioiodine was obtained in the 4 patients tested. Before treatment the uptake varied from 58 to 75 per cent, and during treatment varied from 5 to 13 per cent, as follows:

Patient	*Before treatment*	*During treatment*
H.E.	58%	5.6%
W. J.	67%	13%
E. O.	75%	10%
G. G.	Not obtained	5%

III. *Thyroid iodine content.* Nine glands were examined and, as seen in Table 2, the total iodine content ranged from 18.1 to 71.6 milligrams per cent of wet tissue with an average of 39.1 milligrams per cent, and the protein-bound iodine content ranged from 8.1 to 53.3 milligrams per cent with an average of 22.0 milligrams per cent. The total protein-bound iodine

values of the normal thyroid as reported by Rawson and McArthur (3) averaged 67.2 milligrams and 29.8 milligrams per 100 grams of wet tissue, respectively. Exact composition of the protein-bound iodine in the colloid of our cases has not been determined. Further investigation is necessary to determine the calorigenic activity of this colloid and its other properties.

The serum protein-bound iodine is greatly increased in the patients treated with this drug, despite several washings to eliminate the inorganic

TABLE 2

Patient	Iodine content of fresh thyroid gland	
	Total iodine (mg. % wet tissue)	Protein-bound iodine (mg. % wet tissue)
K.M.	62.0	45.7
G.G.	38.8	23.8
E.D.	59.0	16.9
G.E.	40.0	19.0
M.P.	71.6	53.3
H.C.H.	19.4	11.9
K.A.	24.0	8.1
G.L.	19.1	9.1
W.J.	18.1	10.9
Average	39.1	22.0

iodine. Rapport and Curtis (16) have had the same experience. It is hoped that future investigations will explain the nature of this elevated serum protein-bound iodine.

Degree of goitrogenic action

I. *Changes in thyroid size* were measured by clinical observation. In all the patients observed, the gland remained of the same size or decreased slightly. Furthermore, the gland acquired a characteristic firmness or a tendency toward hardness, during treatment, as seen in Table 3.

II. *Histologic changes.* Of the 15 glands examined, 10 showed follicles which appeared to be well filled with colloid of normal appearance; and the thyroid was avascular and moderately involuted, with a few isolated foci of hyperplasia. The connective tissue seemed to be increased and lymphoid tissue was practically absent (Figs. 1, 2, and 3). These 10 glands (Table 3) were firm and relatively avascular during surgery, and the micro-

histometric studies showed a mean acinar cell height of below 10 micra, with a range from 6.29 to 10.0 micra. One gland (M. O., Table 3) had a mean acinar cell height of 11.2 micra and the histologic appearance was the same as that just described. The other 4 glands were friable and vascular at the time of surgery, and their general histologic structure showed more numerous foci of hyperplasia than in the above cases (Fig. 3); however, the follicles were well filled with colloid and the sections showed little lymphoid tissue or hemorrhage. Their mean acinar cell heights were above 11 micra,

TABLE 3

Patient	Dose of iodo-thiouracil, mg.	No. of days administered	Thyroid gland				M.A.C.H. in micra
			Before operation		Surgical findings		
			Hard	Firm	Friable and vasc.	Firm and rel. avascular	
K.M.	200	30	×			×	6.29±0.23*s.d.±2.3†
	200	71					
	600	40					
G.G.	200	28	×			×	7.5 ±0.30 s.d.±3.0
	400	34					
B.M.A.	200	39	×			×	7.41±0.18 s.d.±1.8
E.O.	200	10	×			×	6.0 ±0.27 s.d.±2.7
	400	12					
G.E.	400	19	×			×	8.2 ±0.23 s.d.±2.3
M.P.	600	13		×		×	8.7 ±0.30 s.d.±3.0
H.C.H.	600	21		×		×	9.0 ±0.27 s.d.±2.7
	100	4					
K.A.	600	34		×		×	9.6 ±0.34 s.d.±3.4
G.L.	600	28		×		×	9.7 ±0.29 s.d.±2.9
P.V.	200	33	×			×	10.0‡±0.16 s.d.±1.6
M.O.	200	49	×			×	11.2 ±0.48 s.d.±4.8
S.V.	200	41	×		×		11.6 ±0.50 s.d.±5.0
W.J.	600	60		×	×		11.7 ±0.36 s.d.±3.6
B.I.	200	29		×	×		11.8 ±0.47 s.d.±4.7
B.D.	200	32	×		×		15.6 ±0.35 s.d.±3.5

* Standard error of the mean.
† Standard deviation.
‡ Toxic nodular goiter. Normal tissue showed 6.17±0.22.

ranging from 11.6 to 15.6 micra. In 16 normal autopsy thyroids examined, the mean acinar cell heights ranged from 3.6 to 7.2 micra, with an average of 6.1 micra.

The differences between sections from the two, three, or four blocks cut from the surgical specimens were surprisingly small. Several instances were within a range of 0.1 micra, whereas the greatest range was 1.2 micra. Further analysis of this variability will appear in a subsequent article.

The dosage used does not seem to have affected these results, but it is worthwhile to observe that among the 4 patients with friable and vascular

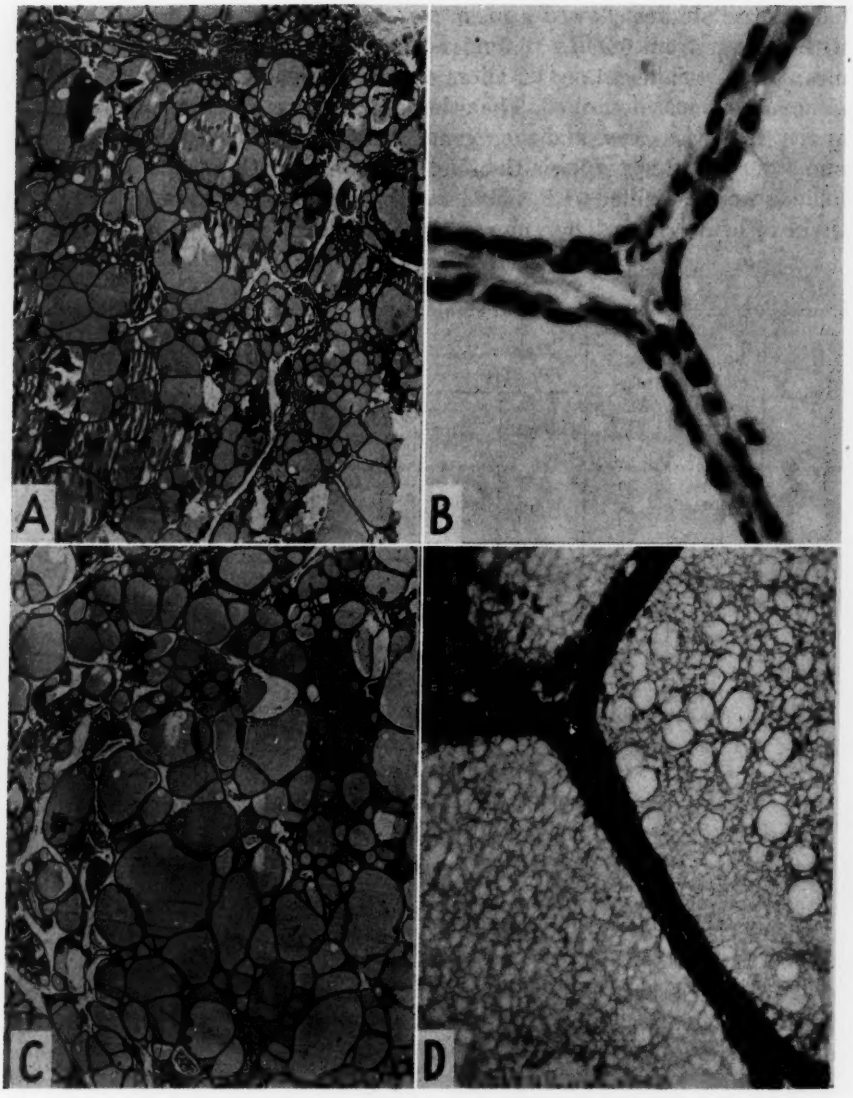

FIG. 1. *A.* Microphotograph, ×30 approx. *B.* Same, oil immersion, ×750 approx. Patient M. P. whose clinical description is given in Figure 4. Gland was firm and relatively avascular; mean acinar cell height, 8.7 micra. *C.* Microphotograph, ×30 approx. *D.* Same, oil immersion, ×750 approx. Patient G. E. whose clinical description is given in Figure 6. Gland was firm and relatively avascular; mean acinar cell height, 8.2 micra.

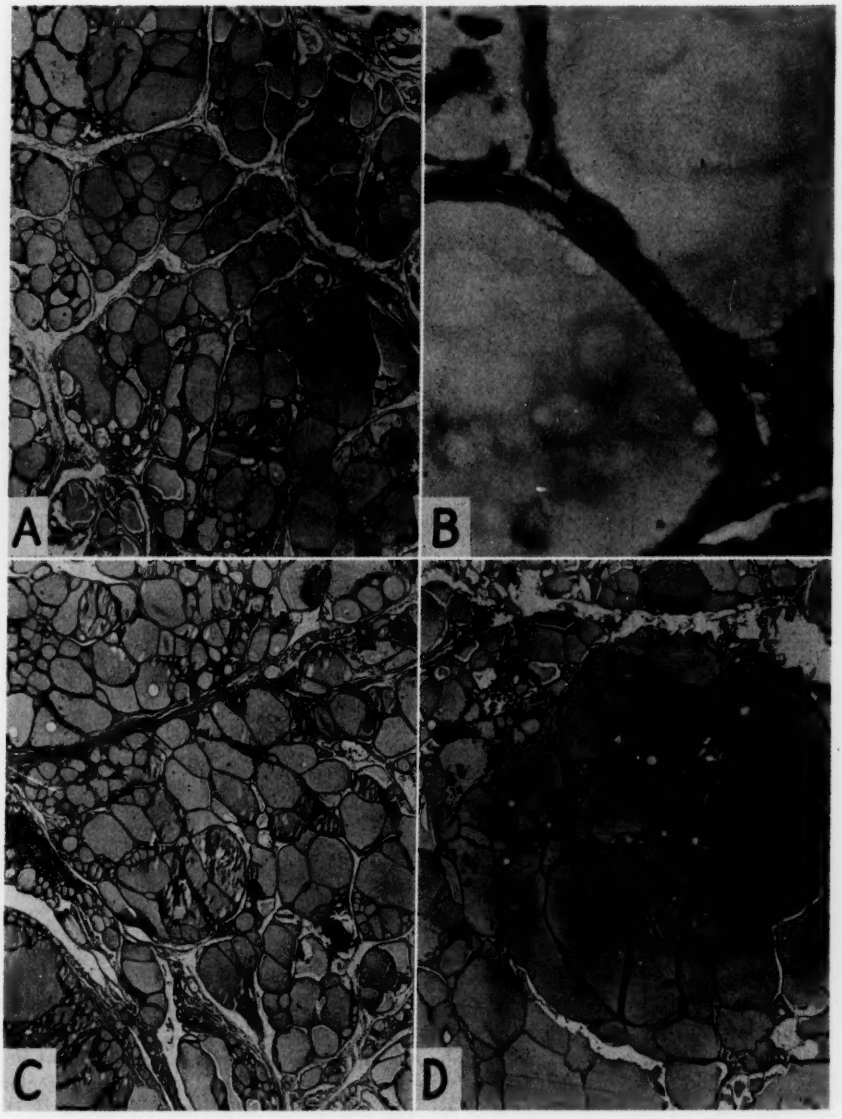

FIG. 2. *A*. Microphotograph, ×30 approx. *B*. Same, oil immersion, ×750 approx. Patient E. O., prepared for surgery with iodo-thiouracil, 200 mg. for ten days and 400 mg. for twelve days. Gland was firm and relatively avascular; mean acinar cell height, 8.0 micra. *C*. Microphotograph, ×30 approx. Patient B. M. A., prepared for surgery with iodo-thiouracil, 200 mg. daily for thirty days. Gland was firm and relatively avascular; mean acinar cell height 7.4 micra. *D*. Microphotograph, ×30 approx. Patient K. M., whose clinical description is given in Figure 5. Gland was firm and relatively avascular; mean acinar cell height, 6.29 micra.

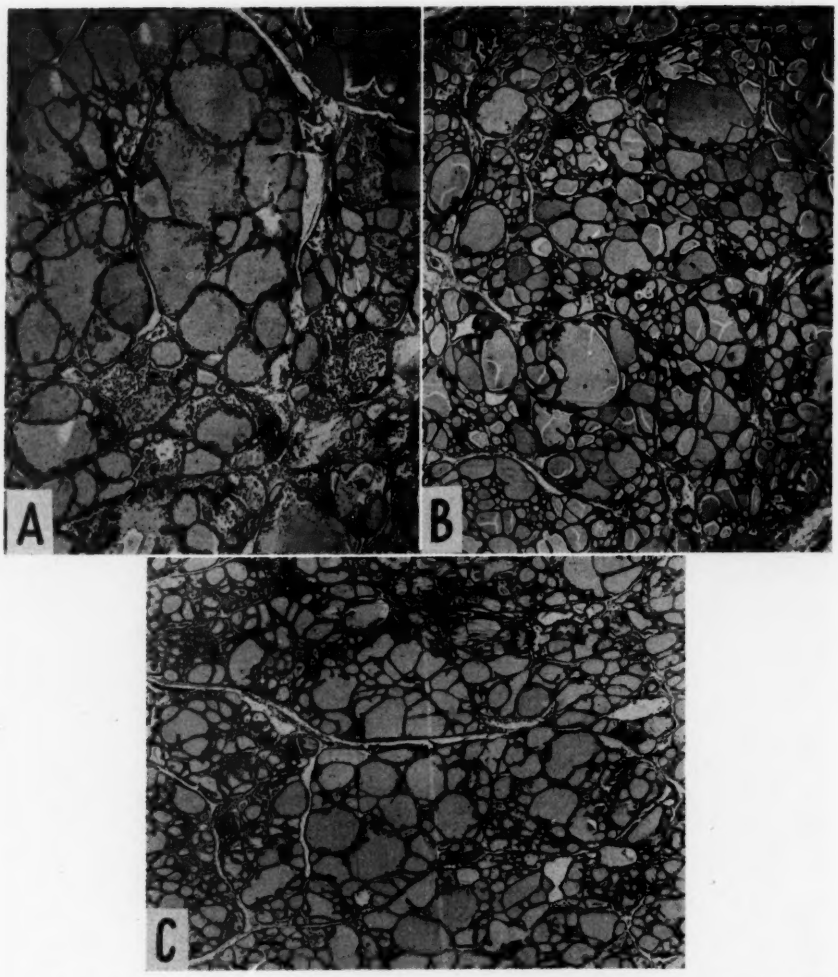

FIG. 3. *A*. Microphotograph, ×30 approx. Patient W. J., prepared for surgery with iodo-thiouracil, 600 mg. for sixty days. Gland was friable and vascular; mean acinar cell height, 11.7 micra. *B*. Microphotograph, ×30 approx. Patient S. V., prepared for surgery with iodo-thiouracil, 200 mg. daily for forty-one days. Gland friable and vascular; mean acinar cell height, 11.6 micra. *C*. Microphotograph, ×30 approx. Patient H. C. H., prepared for surgery with iodo-thiouracil, 600 mg. for twenty-one days and 100 mg. for four days. Gland was firm and relatively avascular; mean acinar cell height, 9 micra.

glands and mean acinar cell heights above 11.6 micra, 3 received a dosage of 200 mg. of iodo-thiouracil daily.

CLINICAL RESULTS

Of the 21 patients prepared, 15 actually underwent surgery; of the 6 patients not operated on, 3 responded slowly and/or incompletely to treatment, 1 was rejected for surgery because of a marked keloid tendency,

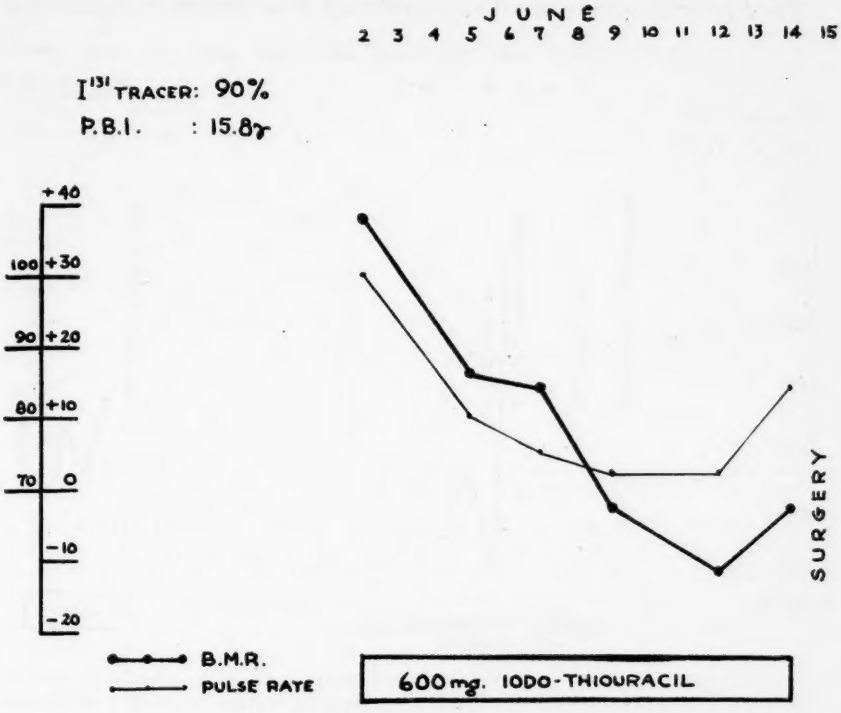

FIG. 4. *M.P.*, aged 29, F. Moderate hyperthyroidism with persistent hypertension. Thyroid gland: 75 Gm., soft. Patient was prepared for surgery in thirteen days with 600 mg. of iodo-thiouracil daily. Maximal clinical response was obtained in eight days. Thyroid gland firm and same size. The blood pressure returned to normal prior to surgery, but several weeks after operation the patient had mild hypertension again. No drug toxicity.

1 stopped medication after being controlled, and in 1 case medication was discontinued because of the development of moderate leukopenia and granulocytopenia.

The postoperative course was smooth in 14 of the 16 patients; all had slight fever for forty-eight hours after surgery and were discharged within three to five days later. Patient G. L. had a post-thyroidectomy hematoma

which was infected and required drainage. Patient P. V. had slight post-thyroidectomy tetany, which was controlled.

In the 2 long-term medical cases, no relapse has occurred to date in nine months of therapy (H. E., Fig. 7), and in two months of therapy (F. G.), respectively.

The 3 patients who received iodo-thiouracil as an adjunct to radioiodine

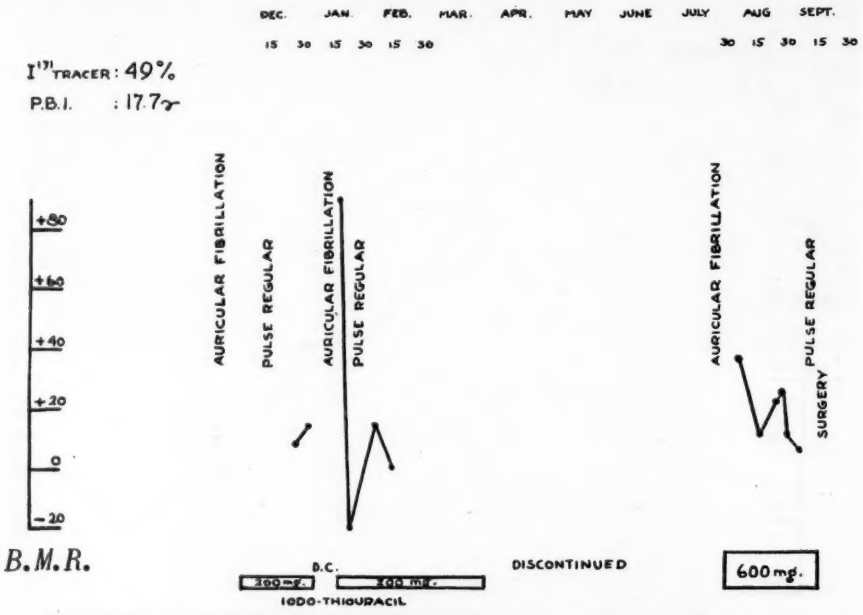

FIG. 5. *K.M.*, aged 48, F. Chronic thyrotoxic heart disease with auricular fibrillation. Thyroid gland: 75–100 Gm., soft. Patient received 3 courses of therapy with iodo-thiouracil. In all of them the auricular fibrillation, which did not respond to digitalis, was converted to sinus rhythm and hyperthyroidism was controlled. Each time medication was stopped, auricular fibrillation and hyperthyroidism recurred. The patient was prepared for surgery with 600 mg. of iodo-thiouracil daily. Thyroid gland firm. No drug toxicity.

therapy seventy-two hours after administration of radioiodine were effectively controlled within four to fifteen days (all marked with an asterisk in Table 1). The effective half-life of a tracer dose of 100 microcuries of radioiodine, which was administered in 5 of our cases seventy-two hours before therapy with iodo-thouracil, was found to be 5.9 ± 0.85 days as compared with a value of 6.2 ± 1.4 days in 20 untreated hyperthyroid patients (17). These results would indicate that iodo-thouracil can be safely administered

as early as seventy-two hours after the dose of radioactive iodine has been given. This is of great assistance for the control of the thyrotoxic symptoms until the radioiodine therapeusis is effective.

The clinical description and diagrammatic evolution of the disease in patients M. P., K. M., and G. E., who had surgery; H. E., who had long-term medical treatment; O. R., who gave an incomplete response to treat-

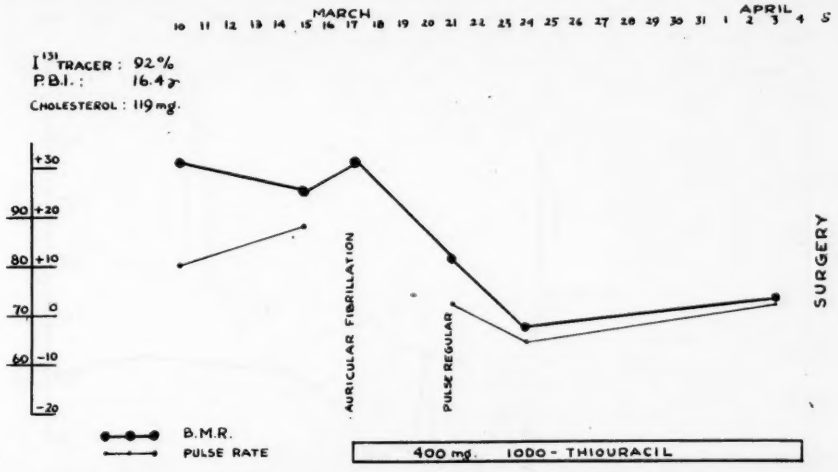

FIG. 6. *G.E.*, aged 48, F. Acute thyrotoxicosis with auricular fibrillation, exophthalmos, and quadriceps weakness. Thyroid gland: 40 Gm., soft. Maximal clinical response on the eighth day of treatment with 400 mg. of iodo-thiouracil. The auricular fibrillation was converted to sinus rhythm, exophthalmos diminished and quadriceps weakness disappeared. Thyroid gland very firm and slightly reduced in size. Thyroidectomy was performed after nineteen days of treatment. No drug toxicity.

ment; and W. G., who manifested leukopenia and granulocytopenia, are given in Figures 4, 5, 6, 7, 8, and 9, respectively.

Side effects. As seen in Figure 9, patient W. G. received 600 mg. of iodo-thiouracil daily for twenty-three consecutive days. Her hyperthyroidism was controlled and she was ready for surgery, when mild leukopenia and granulocytopenia developed on the twenty-third day of treatment. Discontinuance of the drug and administration of penicillin and Lugol's solution restored the white blood cell picture to normal. No agranulocytosis, drug fever, or other sensitiveness to iodo-thiouracil was observed in our series.

Dosage. The dosage of iodo-thiouracil used in our series varied from 50 to 800 mg. daily. In the cases prepared for surgery, it ranged from 200 to 600 mg. daily. In the long-term medical cases, the initial dose was from

200 to 400 mg. daily, and the maintenance dose was 50 mg. daily. It is our impression that doses ranging from 200 to 400 mg. are quite effective in preparing for surgery and initiating medical treatment.

At present an investigation is under way here in a series of patients who are being treated with thiouracil plus potassium iodide in doses equivalent to those used in patients treated with iodo-thiouracil.

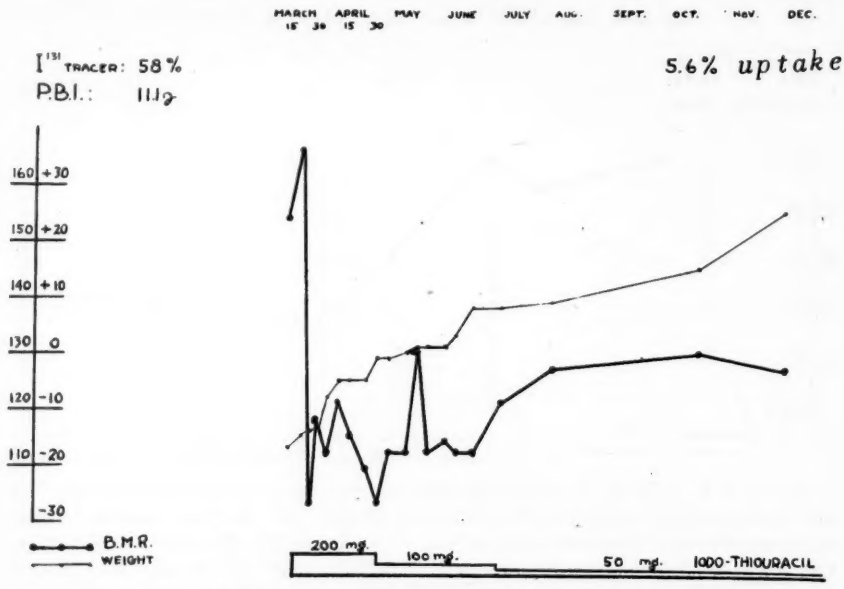

FIG. 7. *H.E.*, aged 74, F. Chronic hyperthyroidism and auricular fibrillation with an apical rate above 140. Thyroid gland: 30 Gm., soft. The maximal clinical response was obtained on the seventh day of treatment with 200 mg. of iodo-thiouracil. Fibrillation persisted, with an apical rate of 72. After forty-seven days on 200 mg., iodo-thiouracil was reduced to 100 mg. daily, and sixty-four days afterwards, to 50 mg. daily as a maintenance dose. To date, in the ninth month of treatment, the BMR is −3 per cent, tracer uptake of I^{131} is 5.6 per cent, the patient has gained 40 pounds, fibrillation persists with an apical rate of 64, and the thyrcid gland is barely palpable. No drug toxicity.

SUMMARY AND CONCLUSIONS

Twenty-six cases of hyperthyroidism have been treated with iodo-thiouracil in doses varying from 50 to 800 mg. daily. In 21 it was as a preparation for surgery; in 3, as an adjunct to radioactive iodine therapy; and in 2, as long-term medical therapy. The maximal clinical response was obtained within eight days in 10 cases, within sixteen days in 7 cases, and within twenty-two days in 5 cases. In 4 cases response was either slower or

incomplete. The optimal dose, as an initial dose for medical management or as preparation for surgery, was between 200 and 400 mg. daily. Further increase of the dose did not improve therapeutic response. Fifty mg. was the medical maintenance dose. Dosage of 600 mg. per day in one case, produced mild leukopenia and granulocytopenia. No other side effects were encountered. Fifteen of the patients had thyroidectomies. At the time of

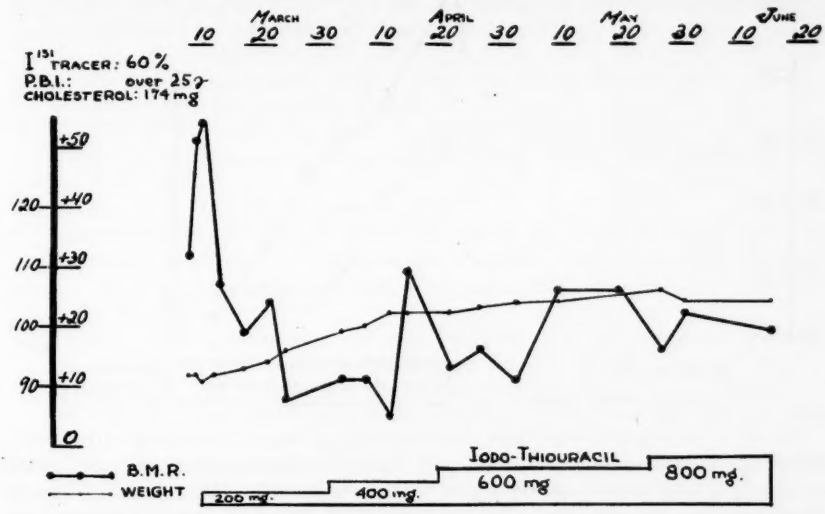

FIG. 8. *O.R.*, aged 20, F. Acute hyperthyroidism. Thyroid gland: 75–100 Gm., medium consistency. Despite the fact that the BMR decreased from +54 to +19 per cent, and the patient gained 14 pounds, the hyperthyroidism remained incompletely controlled after ninety-five days of treatment with iodo-thiouracil in doses progressively increased from 200 to 800 mg. daily. The maximal decrease in BMR was obtained on the thirtieth day of treatment after which there was a slight rise. No drug toxicity.

surgery, 10 of the glands were firm and avascular with mean acinar cell heights of below 10 micra and 1 was firm and avascular with a mean acinar cell height of 11.2 micra; the microscopic picture showed avascularity, follicles with good colloid, a few isolated hyperplastic foci, very little lymphoid tissue, and a tendency toward normal appearance. The other 4 glands were friable and vascular and on examination showed mean acinar cell heights above 11.6 micra; the microscopic study demonstrated follicles with good colloid, but there was an increase in isolated hyperplastic foci and some lymphoid tissue. The glands did not increase in size during treatment. Chemical analysis of the surgical specimens from 9 patients showed an average total iodine content of 39.1 mg. per cent with an average protein-bound iodine content of 22.0 mg. per cent.

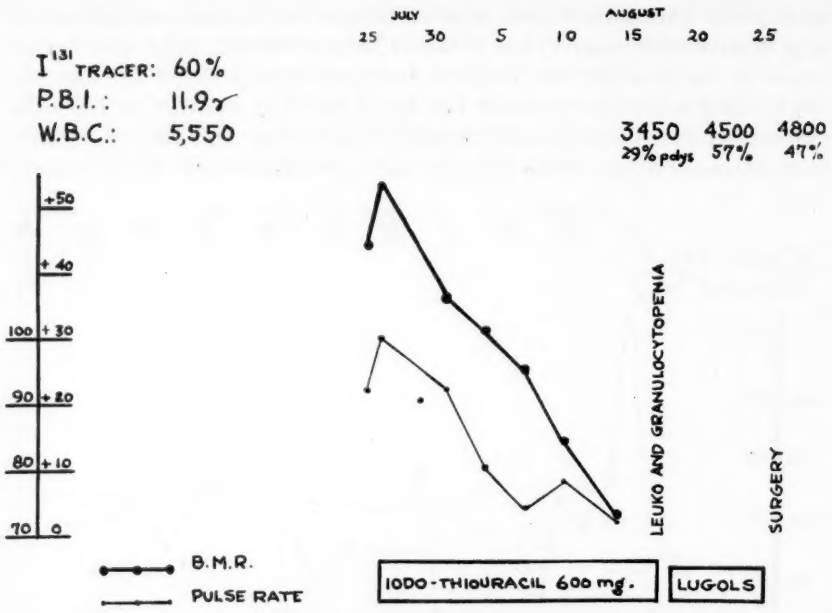

FIG. 9. *W.G.*, aged 46, F. Chronic hyperthyroidism, treated previously with methylthiouracil. Thyroid gland: 60–75 Gm., soft. Maximal clinical response occurred within sixteen days after treatment was begun with iodo-thiouracil, 600 mg. daily. Thyroid gland same size and very hard. On the twenty-third day of treatment leukopenia and granulocytopenia developed and the drug was discontinued. Lugol's solution was then given and the white blood cell count was restored to normal in four days. Thyroidectomy was then performed.

In this small series, iodo-thiouracil showed an effective antithyroid action and no goitrogenicity.

Acknowledgment

We acknowledge, with thanks, the assistance of Mrs. Ingrid Markul, M. S., with the chemical analyses.

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METABOLIC STUDIES WITH I^{131} LABELED THYROID COMPOUNDS: DISTRIBUTION AND EXCRETION OF RADIODIIODOTYROSINE IN HUMAN BEINGS

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PREVIOUS studies of the metabolism of labeled thyroidal substances (1, 2, 3, 4, 5) have suggested that thyroxine is slowly metabolized to at least two substances— inorganic iodide and diiodotyrosine. The further fate of the inorganic iodide liberated from the catabolism of thyroxine is well known, but that of diiodotyrosine is not. Earlier studies dealing with large doses of diiodotyrosine in human subjects indicated that it was decomposed to iodide, since urinary excretion of iodide rose greatly after the administration of diiodotyrosine (6). On the other hand, Salter (7) listed values for the concentration of diiodotyrosine in both erythrocytes and serum of normal persons and of those having various thyroid disorders. The possibilities that diiodotyrosine may exist as a free amino acid in the blood or that it may be accumulated or secreted by the thyroid, merit a reinvestigation of the metabolism of diiodotyrosine in man. We have, therefore, prepared diiodotyrosine in radioactive form (labeled with I^{131}) and have administered the radioactive amino acid to myxedematous patients who were brought to a euthyroid state by therapy with desiccated thyroid. The present paper reports the results of three such studies.

MATERIAL AND METHODS

1. *Subjects.*—Three myxedematous patients being treated effectively with desiccated thyroid volunteered for the investigation of the metabolism of radioactive diiodotyrosine.

Patient A was a woman, aged 58 years, who had had symptoms of myxedema for three years. In addition to the presence of a husky voice, typical facies, edema, intolerance to cold and slowed tendon reflexes, basal metabolic rates were −18 and −24 per cent, and the concentration of cholesterol was 353 mg. per 100 cc. of plasma. The thyroid was not palpable. The patient's response to thyroid therapy was satisfactory, and the study was carried out when she was judged by clinical and laboratory evidence to be in a euthyroid state on a dose of 1½ grains (about 0.1 Gm.) of desiccated thyroid daily.[1] Her weight at the time of the study was 65.45 Kg.

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[1] The thyroid used in these cases was Parke, Davis & Company's strong uncoated thyroid.

Patient B was a man, aged 54 years, who complained that he had experienced fatigue, edema of the ankles, puffiness of the eyelids and hands, slowed speech and intolerance to cold for nine months. His skin and hair were dry, his speech was thick, and his reflexes were sluggish. The basal metabolic rate was −14 per cent, the concentration of cholesterol was 317 mg. per 100 cc. of plasma, and the concentration of protein-bound iodine was 0.3 microgram per 100 cc. of serum. A tiny nodule was present in the right inferior pole of the thyroid. The patient was brought to a euthyroid state within three weeks after the start of thyroid therapy. At the time of administration of radioactive diiodotyrosine, his weight was 80.44 Kg. and he was receiving desiccated thyroid in a dose of 1½ grains (about 0.1 Gm.) daily.

Patient C was a girl, aged 17 years, who complained that she had had progressive weakness for three years. She had not grown for the past six years. The menses were normal. In the few months prior to her appointment at the Mayo Clinic, she had noticed sensitivity to cold. In addition to the dry skin, short stature and husky voice, she had scanty axillary hair. The thyroid was normal in size. The basal metabolic rate was −32 per cent, the concentration of cholesterol was 234 mg. per 100 cc. of plasma, and the concentration of protein-bound iodine was 0.8 microgram per 100 cc. of serum. She made a good response to thyroid therapy. At the time of the study, her weight was 50.15 Kg. She was receiving 1 grain (about 0.065 Gm.) of desiccated thyroid daily.

2. *Preparation of radioactive diiodotyrosine.*—Radioactive diiodotyrosine was prepared by exchange. In a glass-stoppered test tube, 0.1 cc. of a stock solution of diiodotyrosine containing 5 mg. of diiodotyrosine per cc. was heated to 90° C. and mixed with radioactive iodine. The radioiodide was added in as small a volume as possible, the exact amount of radioiodide added being determined by the desired specific activity of the final product. In small runs, the amount of radioiodine used was 100 microcuries. The glass-stoppered tube was placed in a boiling water bath for five minutes. At the end of this time, 0.5 cc. of the stock diiodotyrosine solution was added to the mixture. The mixture was transferred to a 15-cc. centrifuge tube and placed on ice for at least three hours. The crop of cryst ls which formed was centrifuged and washed three times with 2 cc. of distilled water at 0° C. The recovery of I^{131} as measured by the proportion of the amount of I^{131} exchanged, to the initial I^{131} used, varied from 10 to 80 per cent.[2] To prepare radioactive diiodotyrosine with a high specific activity, it was necessary only to modify the procedure by the addition of sufficiently larger amounts of NaI^{131}.

Radioactive diiodotyrosine with high specific activity (between 300 and 400 microcuries of I^{131} per mg. of diiodotyrosine) was prepared for us by this procedure in the Abbott Laboratories, North Chicago, Illinois, through the kindness of Dr. D. Tabern and Mr. R. Gleason. The radioactive di-

[2] No attempt was made to control accurately the conditions of the final reacting mixture such as pH, ionic strength and ratio of reactants, because of lack of equipment for working properly with small volumes of intensely radioactive materials.

iodotyrosine was dissolved in dilute hydrochloric acid at pH 2 and filtered to obtain a sterile solution. The presence of only one I^{131}-containing component, namely, diiodotyrosine, was established both by solubility determinations and by chromatographic analysis.

For administration to patients, an aliquot of the solution containing 500 microcuries of radioactive diiodotyrosine was removed sterilely and diluted to a volume of 10 cc. with sterile saline solution. A dose of 300 microcuries (usually a volume of 6 cc.) was injected intravenously into each of 3 patients. An aliquot of the dose (about 50 microcuries) was transferred to a 250-cc. volumetric flask and, after the addition of 25 mg. of nonradioactive diiodotyrosine as carrier, was made up to mark with distilled water. Measured amounts of this standard solution were removed from time to time and counted simultaneously with samples of blood, urine and feces. The amounts of material that each patient received were as follows: Patient A, 2.0 mg. of diiodotyrosine labeled with 300 microcuries of I^{131}; Patient B, 2.1 mg. of diiodotyrosine labeled with 318 microcuries of I^{131}; Patient C, 1.0 mg. of diiodotyrosine labeled with 300 microcuries of I^{131}.

3. *Procedure on subjects.*—After injection of the radioactive diiodotyrosine into an antecubital vein, 10 to 20 cc. of blood was withdrawn at approximately fifteen, thirty, forty-five and sixty minutes, thereafter hourly for five hours and finally at eight, twenty-four, thirty-six and forty-eight hours. The blood was allowed to clot in order to obtain serum. At intervals of two hours during the first twelve hours and thereafter twice daily, urine was collected with the precautions described by Keating *et al.* (8). Feces were collected whenever passed during the three-day interval of study. At approximately hourly intervals for the first eight hours, and thereafter twice daily, *in vivo* counts were obtained over the neck, thigh, and right and left epigastrium according to the methods described by Luellen *et al.* (9).

4. *Fractionation procedures.* a. Blood.—The various samples of blood were centrifuged to obtain serum. Two 1-cc. samples of serum were removed, the first for the determination of total I^{131} and the second for fractionation. To 1 cc. of the serum in a 40-cc. centrifuge tube were added 9 cc. of saline solution and 0.1 cc. of a 1.0 per cent solution each of thyroxine, diiodotyrosine and sodium iodide, respectively, as carriers. A drop of Congo red indicator solution was added and, with a dropping pipet, 68 per cent trichloracetic acid was added with stirring until a blue color developed. After centrifugation, the clear supernatant solution was transferred to a 40-cc. centrifuge tube containing 10 drops of 40 per cent solution of sodium hydroxide and set aside. The precipitate was taken up in 1 to 2 cc. of water and dissolved with the addition of one or more drops of 40 per cent solution of sodium hydroxide. Distilled water was added to bring the total volume

up to about 8 cc. The precipitation procedure with 68 per cent trichloracetic acid was carried out again. After centrifugation, the supernatant liquid was combined with the corresponding supernatant liquid of the first precipitation and made alkaline to phenolphthalein. The precipitate was dissolved in 3 to 5 cc. of water with the aid of base, and the radioactivity was determined. This precipitate represented the thyroxine fraction.

The combined supernatant solutions were acidified to Congo red with 50 per cent nitric acid. With stirring, 0.4 cc. of 10 per cent solution of silver

TABLE 1. EFFICIENCY OF THE FRACTIONATION PROCEDURE*

Labeled material added		Mean percentage recovery of added labeled materials in the iodide, thyroxine and diiodotyrosine fractions		
		Iodide	Thyroxine	Diiodotyrosine
Iodide	Urine	96.46	1.67	1.87
	Serum	95.46	3.73	0.81
Thyroxine	Urine	5.03	93.36	1.61
	Serum	3.72	95.08	1.20
Diiodotyrosine	Urine	2.79	3.82	93.39
	Serum	4.24	4.44	91.32

* The values given represent the percentage of each fraction as determined by dividing the counts of each fraction by the sum of the counts for all three fractions. Losses on glassware and during manipulation are negligible, usually less than 1 per cent, but occasionally as high as 3 per cent.

nitrate in 2 per cent nitric acid was added. The solution was immediately centrifuged, and the supernatant fluid was transferred to flasks for determination of the I^{131}. This fraction represents the diiodotyrosine fraction. The silver precipitate (the iodide fraction) was discarded, since it was not possible to determine the I^{131} in this fraction because of the presence of silver which prevents quantitative distillation of the iodine.

The I^{131} of the two fractions (thyroxine and diiodotyrosine), together with 1 cc. of the unfractionated serum and of an aliquot of the pilot, was determined by a digestion and distillation procedure (10). The I^{131} of whole serum being known, and the I^{131} in the thyroxine and diiodotyrosine fractions being known, the I^{131} in the iodide fraction was calculated by difference. The efficiency of this fractionation procedure is given in Table 1, which shows that 90 per cent or more of each fraction is recovered and that

no appreciable exchange or contamination occurred. It should be emphasized at this point that the so-called thyroxine and diiodotyrosine fractions represent free thyroxine and free diiodotyrosine and also that they might contain, in small quantities, other I^{131} compounds that are so far unidentified. It is also clear that in addition, the thyroxine fraction is the protein-bound fraction of blood.

b. Urine.—The volume of each sample of urine was determined, and 10 cc. was withdrawn from each sample for counting. Together with a suitable aliquot of the pilot made up to 10 cc., the samples of urine were counted at a fixed position in a gamma ray counter. From the total volume, the total amount of I^{131} in each sample of urine was readily determined as percentage of the administered dose.

In the fractionation procedure, sufficient urine from each sample was withdrawn so that 0.5 to 1 microcurie of I^{131} was present. When less than 10 cc. of urine was required to obtain this amount of radioactivity, saline solution was added to make up to 10 cc. To each 40-cc. centrifuge tube containing 10 cc. of urine, or of urine diluted with saline solution, 1 mg. of thyroxine, diiodotyrosine and sodium iodide, respectively, was added as carrier. Two-tenths cubic centimeter of normal serum albumin (Cutter Laboratories) was added to each tube, followed by a drop of Congo red indicator. Sixty-eight per cent trichloracetic acid was then added with a dropping pipet until a blue color was obtained, and the mixture was centrifuged. The supernatant solution was poured into another 40-cc. centrifuge tube containing 10 drops of 40 per cent solution of sodium hydroxide and set aside. The precipitate was dissolved by the addition of 5 cc. of water and 1 or 2 drops of 40 per cent solution of sodium hydroxide until complete solution occurred. Trichloracetic acid was added until the indicator turned blue, and the mixture was centrifuged again. The supernatant was combined with the supernatant of the first precipitation. The precipitate was dissolved by the addition of water and base and made up to a volume of 20 cc. This represents the thyroxine fraction, containing free and protein-bound thyroxine.

To the combined supernatant fluid, 50 per cent solution of nitric acid was added until the solution was acid to Congo red. With stirring, 0.4 cc. of 10 per cent solution of silver nitrate in 2 per cent nitric acid was added, and the solution was centrifuged. The supernatant fluid, made up to 20 cc. in volume, represents the diiodotyrosine fraction. The precipitates were stirred with a few cubic centimeters of 60 per cent solution of sodium thiosulfate until partial solution occurred and then were made up to 20 cc. This is the iodide fraction. Pilots of the original dose and urine equal to the amount of urine taken for fractionation were also made up to 20 cc. All the samples were counted simultaneously at a fixed position

in a gamma ray counter. Again it must be pointed out that only the free forms (except thyroxine) of these compounds are thus determined and that unknown metabolites labeled with I^{131} can be present in these so-called thyroxine and diiodotyrosine fractions. The extent of the accuracy of these determinations is given in Table 1.

c. Feces.—The feces were collected and weighed. After a uniform suspension had been made, a small portion was withdrawn, weighed and placed in an 80-cc. tube. This sample was counted together with a suitable pilot of the dose, and the total amount of I^{131} in the stool was calculated.

TABLE 2. I^{131} IN SERUM OF 3 PATIENTS (A, B, C) AFTER ADMINISTRATION OF RADIODIIODOTYROSINE

Time, hrs.	Per cent of dose/liter			Per cent of serum I^{131} as								
				Thyroxine			Diiodotyrosine			Iodide		
	A	B	C	A	B	C	A	B	C	A	B	C
15*		12.3			5.3			77.6			17.1	
20*	10.91		20.3	4.0		5.66	76.3		79.6	19.7		14.74
30*		9.7			5.0			64.4			30.6	
45*			11.28			5.35			64.12			30.53
1	7.51	7.3	8.99	4.0	4.9	3.28	63.0	52.6	45.81	33.0	42.5	50.91
2	4.40	4.97	6.67	4.0	0.8	3.73	32.2	36.0	29.88	63.8	63.2	66.39
3		4.1	5.05		3.7	3.27		24.2	20.7		72.1	76.03
4	3.59	3.6	4.65	4.0	5.2	2.20	20.2	17.8	11.89	75.8	77.0	85.91
6	2.60	2.9	3.05	4.0		3.03	16.0		8.77	80.0		88.2
8	1.94	2.5	2.76		5.1	7.79		6.7	5.05		88.2	87.16
10	1.44											
25	1.15	0.7										
33	0.31											
49		0.3										

* Minutes.

5. *Counting equipment.*—The gamma ray counting apparatus used in these studies consisted of a gamma ray counter (Texas Company, Houston, Texas) connected to an autoscaler (Tracer Laboratory, Boston, Massachusetts). The characteristics of the counter were such that a negligible correction for resolving time was necessary with fewer than 1,000 counts per second. Regardless of the volume of any sample, the counter proved very satisfactory for rapid determinations of I^{131} within a range of 0.05 to 2 microcuries.

RESULTS

The data, summarized in Tables 2, 3, and 4, indicate that the observations were quite similar in all 3 cases. Consequently, the curves for only Patient A will be given. In Figure 1 are shown concentrations of various fractions of radioiodine in serum plotted against time (Patient A) after intravenous injection of radiodiiodotyrosine. The concentration of diiodotyrosine in serum diminished with great rapidity during the first two

TABLE 3. URINARY EXCRETION OF I^{131} AFTER ADMINISTRATION OF RADIODIIODOTYROSINE IN 3 PATIENTS (A, B, C)

Time, hrs.	Accumulated per cent of dose			Per cent of urinary I^{131} as								
				Thyroxine			Diiodotyrosine			Iodide		
	A	B	C	A	B	C	A	B	C	A	B	C
2	19.40	23.18	20.52	3.35	1.66	0.72	13.53	11.73	6.98	83.12	86.61	92.30
4	28.76	30.72	33.36	2.23	1.17	0.68	10.63	4.31	4.48	87.14	94.52	94.84
6	43.78	39.14	44.31	1.98	1.32	1.63	7.29	3.36	4.84	90.73	95.32	93.53
8	52.99	47.09	51.62	3.19	1.17	0.47	5.06	2.13	2.70	91.75	96.70	96.83
10	57.16	53.79	57.68	4.79	0.90	3.15	3.94	1.40	2.23	91.27	97.70	94.62
12	64.32	59.12	63.08	3.44	1.54	0.79	3.85	1.51	1.72	92.71	96.95	97.49
18	71.34	69.25	73.30	2.53	2.56	0.85	3.12	1.71	1.52	94.35	95.73	97.63
24–25	80.73	77.95	80.91	2.57	1.31	0.68	2.51	1.25	1.30	94.92	97.44	98.02
30	84.79			2.87			2.82			94.31		
36	86.77	85.73	90.72	3.56	1.50	0.51	3.39	1.65	1.16	93.05	96.85	98.33
42	87.00			2.03			4.71			93.26		
48	88.24	90.03	95.53	3.07	1.44	0.00	5.27	1.68	2.13	91.66	96.88	97.87
72	89.69	94.30			1.77			2.74			95.49	
96	90.39											

hours, but less rapidly thereafter. A considerable inorganic iodide fraction appeared in the first sample measure and increased slowly, leveling off between the second and the fourth hour. Thereafter, it diminished slowly. The thyroxine I^{131} fraction remained very small and appeared to follow the curve of total radioactivity.

Curves indicating the quantities of the same fractions excreted in urine are shown in Figure 2. A small proportion of the dose (5.8 per cent in forty-eight hours) appeared in the urine as diiodotyrosine. Most of this was excreted within six hours, and thereafter only minute amounts of radioactivity were present in this fraction. Inorganic iodide accounted for the

TABLE 4. *In vivo* DETERMINATIONS OF I^{131} AFTER ADMINISTRATION OF RADIODIIODOTYROSINE IN 3 PATIENTS (A, B, C)

Time, hrs.	Counts per second per 100 microcuries of I^{131}											
	Neck			Left epigastrium			Right epigastrium			Left thigh		
	A	B	C	A	B	C	A	B	C	A	B	C
15*		2.38			3.09			2.46			0.93	
30*			3.23			4.45			3.79			1.31
1	2.23		3.02	2.96		4.54	3.10		4.03	0.81		1.13
2	1.55	1.76	2.50	2.56	2.54	3.42	2.62	2.11	3.42	0.66	0.74	1.13
3		2.63	2.63		2.24	3.47		1.46	3.07		0.75	0.88
4	1.45			1.89			2.64			0.44		
6	1.18	1.22	1.92	1.78	2.37	3.77	2.13	1.68	2.69	0.46	0.65	0.75
8	0.80	1.02	1.38	1.34	1.95	3.15	1.89	1.31	2.81	0.31	0.50	0.70
25	0.19	0.43	0.78	0.39	0.75	0.90	0.51	0.50	0.82	0.04	0.18	0.30
33	0.12			0.24			0.41			0.01		
49		0.16			0.39			0.22			0.09	

* Minutes.

greater part of the urinary radioactivity (75 per cent of the dose in forty-eight hours) and continued to be excreted in substantial quantities throughout the period of observation and after the excretion of diiodotyrosine was virtually complete. The urinary radiothyroxine fraction remained very small and appeared to follow the curve of total radioactivity.

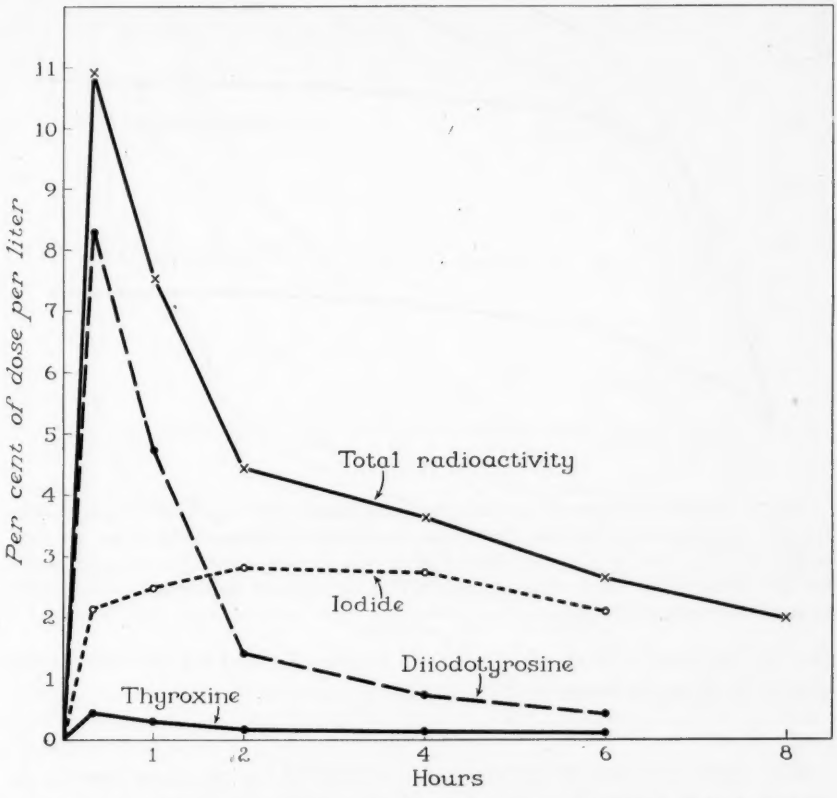

FIG. 1. Radioiodine fractions in serum after intravenous injection of radiodiiodotyrosine (Patient A). The precipitous disappearance of diiodotyrosine from serum is measured by the equally swift growth of the inorganic iodide fraction.

In vivo studies failed to disclose significant localization of radioactivity anywhere in the body. In the four sites followed serially with an *in vivo* counter (Fig. 3) radioactivity decreased rapidly and exponentially, and paralleled the curve for total radioactivity in the serum.

The feces contained comparatively minute amounts of radioactivity (2.3 per cent of the dose in seventy-eight hours in Patient A; 7.5 per

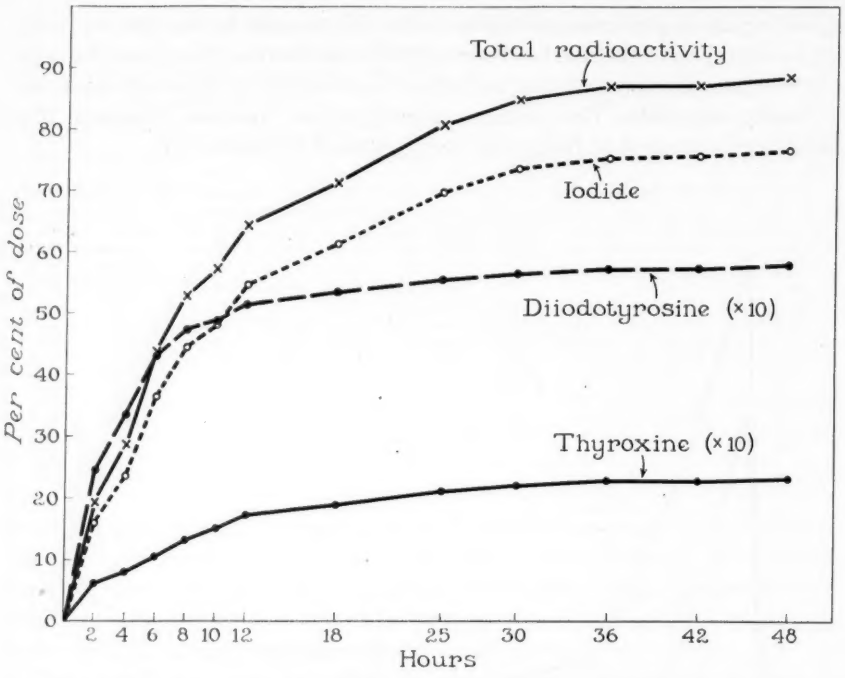

FIG. 2. Urinary excretion of radioiodine after intravenous injection of radiodiiodotyrosine (Patient A). Note that the values for diiodotyrosine and thyroxine are multiplied by 10 in order to provide a more legible curve. Urinary excretion of radiodiiodotyrosine stops within six to eight hours, whereas excretion of radioiodide continues for more than twenty-four hours.

cent of the dose in forty-eight hours in Patient B; and 0.3 per cent of the dose in forty-eight hours in Patient C).

DISCUSSION

It is apparent that diiodotyrosine is converted for the most part to inorganic iodide, which is excreted in the urine. This result was anticipated from earlier studies with diiodotyrosine in pharmacologic quantities. It was on this account that athyreotic persons were selected for this study, since we anticipated that a functioning thyroid might add ruinous complexities to the study.

The extraordinary velocity with which diiodotyrosine disappeared from the blood and urine, to be replaced by inorganic iodide, was unexpected. The possibility had to be entertained, therefore, that this process might represent exchange occurring *in vivo* between diiodotyrosine-I^{131} and in-

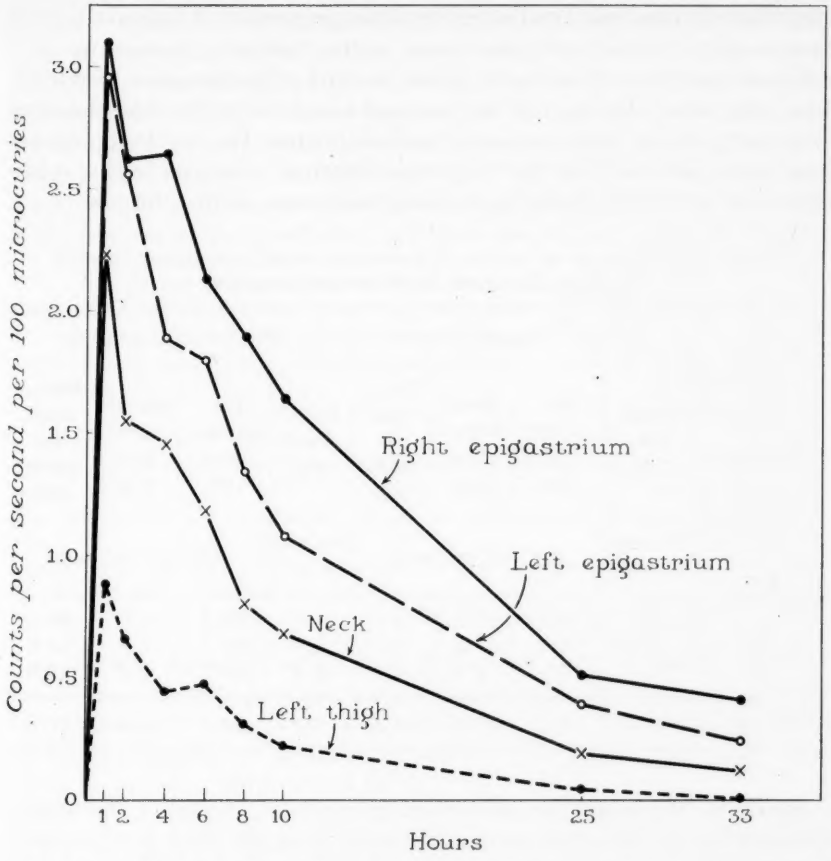

FIG. 3. Serial *in vivo* observations (Patient A). The curves of radioactivity in all organs resembled that of total radioactivity in serum and indicate no area of selective localization.

organic iodide-I^{127}. Such exchange had been described by Miller and others (11) *in vitro* and was employed by us in the preparation of radiodiiodotyrosine. We have been unable to induce molecular exchange under conditions of temperature and pH corresponding to *in vivo* conditions. Incubation of radiodiiodotyrosine with inorganic iodide in blood or in urine for varying periods up to three days failed to disclose any evidence of such exchange. For these reasons, we feel that the phenomena recorded in this report probably represent biologic, not molecular, events.

The "thyroxine curves" in both blood and urine of all 3 patients paral-

leled the curves of total radioactivity. The proportion of radioactivity of each sample of blood and urine found in the thyroxine fraction was not different from the occlusive error of the method of fractionation (Table 1). The behavior of this fraction in these cases is quite unlike that observed in similar patients after injection of radiothyroxine. The conclusion, therefore, seems justified that the "thyroxine fraction" observed in this study represents artefact and that no radiothyroxine was, in fact, formed.

TABLE 5. COMPARISON OF KINETIC BEHAVIOR OF IODIDE AND DIIODOTYROSINE IN URINE AFTER INJECTION OF DIIODOTYROSINE

Patient	Urinary inorganic iodide				Urinary diiodotyrosine			
	Renal fraction	Disappearance rate	Renal excretion rate	Extrarenal disposal rate	Renal fraction	Disappearance rate	Renal excretion rate	Extrarenal disposal rate
	Per cent of dose	Per cent per hour			Per cent of dose	Per cent per hour		
A	76.8	10.5	8.1	2.4	4.1	31.4	1.3	30.1
B	84.0	8.9	7.5	1.4	2.9	60.8	1.8	59.0
C	91.1	8.1	7.4	0.7	2.6	34.7	0.9	33.8
Average	*84.0*	*9.2*	*7.7*	*1.5*	*3.2*	*42.3*	*1.3*	*41.0*

Similarly, the proportion of urinary radioactivity found in the diiodotyrosine fraction of urine samples collected from the sixth to the eighth hour or subsequently, corresponded to blank or occlusive values obtained in the recovery studies (Table 1). All diiodotyrosine values in urine samples prior to the sixth hour exceeded such blank values. It may be concluded that all or substantially all of the diiodotyrosine to be excreted in the urine appeared within the first six hours and that the small amount of radioactivity in the diiodotyrosine fraction after that time should be interpreted as artefact. The diiodotyrosine levels in serum during the first six hours were all in a highly significant range, but the slope of the serum curve makes it reasonable to assume that it would reach extinction values soon after six hours. In analyzing the various serum and urine curves, we corrected the observed concentrations for such occlusive errors. However, values derived from corrected data did not differ greatly from values calculated directly from uncorrected data.

Urinary excretion curves for both inorganic iodide and diiodotyrosine

(Fig. 2) appeared to be approximately exponential in form. Accordingly, they were analyzed in the manner previously described (4, 5) for urinary radioiodide after administration of inorganic iodide. The values derived are summarized in Table 5. Renal fraction and disappearance rate represent the estimated parameters of the urinary curves, defined as the asymptotic value of the urinary curve expressed as per cent of dose, and the proportional rate of disappearance from blood, respectively. Renal excretion rate is the product of the parameters and gives the proportional rate of excre-

TABLE 6. RENAL CLEARANCE OF IODIDE AND DIIODOTYROSINE

Patient	Renal clearance, cc. serum cleared per minute		Ratio iodide: diiodotyrosine clearance
	Inorganic iodide	Diiodotyrosine	
A	34.2	6.5	5.3
B	22.3	3.7	6.0
C	26.4	5.4	4.9
Average	*27.6*	*5.2*	*5.4*

tion in urine, expressed as per cent of dose per hour. Extrarenal disposal rate is the difference between renal excretion rate and disappearance rate and measures the rate of disposal to all sites other than urine. For more detailed description of these terms and their derivation the reader is referred to previous publications (4, 5).

It is rather remarkable that the curve depicting excretion of inorganic radioiodide in urine after the intravenous administration of radiodiiodotyrosine should be so similar in all respects to that observed after administration of a tracer dose of inorganic radioiodide. The extent of the similarity may be judged by comparing the values shown in Table 5 with the following, representing the mean values in 46 cases of myxedema (12): renal fraction 81.7 per cent, disappearance rate 6.6 per cent, renal excretion rate 5.4 per cent, extrarenal disposal rate 1.2 per cent. The values representing the behavior of diiodotyrosine are quite different: the renal fraction is less than 4 per cent of the inorganic iodide value; the disappearance rate is about five times the iodide value. Renal excretion of diiodotyrosine, as indicated by renal excretion rate, is about a sixth the rate of iodide excretion; extrarenal disposal rate of diiodotyrosine is more than twenty-seven times that of iodide.

The renal excretion of diiodotyrosine may be further defined by comparing the renal clearance of that substance with that of inorganic iodide. The

clearances in Table 6 were calculated in the conventional manner (UV/B) using urinary and serum data for the interval from the second to the sixth hour. The values for inorganic iodide clearance correspond to those obtained from radioiodide tracers in euthyroid persons (average 32 cc./min.), whereas those for diiodotyrosine clearance are less than a fifth of these values.

When serum radiodiiodotyrosine is plotted against time on semilog paper, a single straight line cannot be made to fit the points even when due correction is made for errors resulting from occlusion. The curve in all 3 cases fits a double exponential function similar to that described for intravenous-

TABLE 7. APPROXIMATE VOLUME OF DISTRIBUTION OF DIIODOTYROSINE

Patient	Initial phase		Secondary phase	
	Liters	cc./kilogram	Liters	cc./kilogram
A	6.7	102	37	570
B	6.7	84	41	510
C	5.4	69	25	500
Average	*6.3*	*85*	*34*	*530*

ly administered mannitol or radiothyroxine. Values derived from analysis of the serum curve provide roughly quantitative information about several metabolic events: 1) The initial volume of distribution[3] of radiodiiodotyrosine in these cases (Table 7) averages 85 cc. per kilogram, a value corresponding closely to whole blood volume. 2) There was an initial rapid decrease of radiodiiodotyrosine from serum for approximately two hours. This apparently represents diffusion out of blood into a much larger volume of distribution (average about 34 liters, or 530 cc. per kilogram). The rate of diffusion represented by the slope of this initial curve varied from 2 to 4 per cent per minute (half-value time seventeen to thirty-six minutes). 3) After two hours, there becomes evident a slower rate of disappearance of radiodiiodotyrosine from this second larger volume. This rate appears to correspond to that derived from the urinary diiodotyrosine curves (Table 8). Such a relationship may be construed as indicating that diiodotyrosine is excreted in the urine and disposed of elsewhere (for exam-

[3] Volume of distribution is here defined as the volume which would be occupied by the material in the organism, assuming a concentration uniformly the same as that observed in serum.

ple, by deiodination) at a constant rate proportional to its serum concentration.

Similar inspection of the curve of serum inorganic iodide (Fig. 1) is less informative, owing to the comparatively inadequate number of observa-

TABLE 8. COMPARISON OF RATE CONSTANTS DERIVED FROM SERUM AND URINE

Patient	Diiodotyrosine		Inorganic iodide	
	Per cent per hour Disappearance rate as estimated			
	From urine	From serum	From urine	From serum
A	31.4	31.5	10.5	12.3
B	60.8	46.1	8.9	6.7
C	34.7	43.3	8.1	10.8
Average	*42.3*	*40.3*	*9.2*	*9.9*

tions compared with the greater complexity of the relationships which must be assumed. The curve involves at least two components: an initial phase representing the accumulation or growth of a serum inorganic iodide fraction and its diffusion; and the second phase, a simple exponential disappearance curve representing the disappearance of inorganic iodide from the blood and body fluids, principally into the urine. It is reassuring to observe that the rate estimated from the second component of the serum curve agrees satisfactorily with the disappearance rate as calculated from the urine curve (Table 8).

The total radioactivity recovered in urine and feces in this study represented the same proportion of the dose as is ordinarily recorded after administration of labeled inorganic iodide to athyreotic subjects. The conclusion, therefore, seems warranted that diiodotyrosine is disposed of entirely by deiodination, except for the very small proportion which escapes deiodination by being excreted intact in the urine. Since *in vivo* observations did not indicate any selective localization of the labeled diiodotyrosine anywhere in the body, the exact location where deiodination occurs remains a matter for conjecture. However, if one considers the rapidity with which iodide is released from diiodotyrosine, it would seem improbable that localization or retention of labeled diiodotyrosine at the site of the deiodinating reaction could be demonstrated.

If the premise is accepted that deiodination is the only significant alternative route of disposal for injected diiodotyrosine other than urinary excre-

tion, then the rate of deiodination may be represented, at least as a first approximation, by the values listed in Table 5, column 9, as extrarenal disposal rate of diiodotyrosine. One can estimate by simple proportionality that these values indicate that approximately 150 to 200 cc. of serum are cleared of their diiodotyrosine content by deiodination per minute. Since this corresponds to 300 to 500 cc. of whole blood per minute, it is evident that the process must either occur diffusely, as in the muscles, or in some organ with a very large blood flow, such as the liver.

SUMMARY

The metabolism of radioactive diiodotyrosine was studied in 3 myxedematous patients by administering intravenously from 1 to 2 mg. of diiodotyrosine labeled with I^{131}. Serial fractionation procedures were performed on samples of blood and urine, serial *in vivo* observations of radioactivity were made, and the total fecal excretion of I^{131} was determined. Radioactive diiodotyrosine disappeared rapidly from the blood and was replaced by radioactive iodide. The initial volume of distribution of diiodotyrosine corresponded to blood volume (85 cc./Kg.), but within two hours, diffusion into a much larger compartment corresponding to 530 cc./Kg. occurred. Only small quantities of radiodiiodotyrosine were excreted in the urine, and this only during the first six hours following injection. The greatest proportion of the urinary I^{131} consisted of iodide during the first few hours, and subsequent to this time all of the urinary I^{131} was in the form of iodide. The renal clearance of radiodiiodotyrosine was 5.2 cc. per minute, a fifth of the renal clearance of the radioiodide liberated from it. An average of 3 per cent of the administered radioactivity was found in the feces. No localization of I^{131} occurred in any of the sites measured by an *in vivo* counter.

Acknowledgments

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COMPARISON OF A SINGLE G-M TUBE METHOD AND A MULTITUBE METHOD OF ASSAYING I^{131} UPTAKE BY THE HUMAN THYROID

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THE measurement of the accumulation of radioactive iodine by the human thyroid gland has been reported to be the most accurate assay of thyroid function (1, 2). The technique of direct measurement of the accumulation of I^{131} by external counting over the thyroid gland using a single Geiger-Mueller (G-M) tube has been employed extensively. However, Freedberg *et al.* (3) have pointed out that with this method, errors in recorded count due to variation in gland size and location are large. They concluded that the need remained for a direct simple quantitative technique which would not be affected by minor differences in the size, shape, and location of the gland. Devising a new method by using four G-M tubes connected in a parallel electric circuit and arranged circularly in a horizontal plane, they reported that this technique was more accurate than the single-tube method. Hertz *et al.* (4) have also reported upon the use of the four-tube multicounter.

This present study was initiated to compare the two methods, the single-tube or the four-tube method, in assaying the content of radioactivity in the human thyroid gland.

METHODS

Multicounter method. The multicounter[1] employed was an apparatus which consisted of four G-M tubes[2] in a parallel electric circuit connected to a single scaler. The tubes were shielded by lead, $1\frac{7}{8}$ inches in thickness, with the exception of a narrow slit directed toward the center of the circle. This slit measured 4 inches long by $1\frac{1}{2}$ inches wide, tapering inward to a width of 1 inch. Each of the four tubes was covered with two thicknesses of lead foil in order to increase efficiency. By placing a source of radiation in the center of this circle, the radiation was recorded simultaneously by each of the four tubes and the summation was recorded by the scaler.

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[1] The multicounter apparatus of Hertz *et al.* (4) was loaned to us by the Atomic Instrument Company of Boston, Massachusetts.

[2] G-M tube Model 1B85, Victoreen Instrument Co., Cleveland, Ohio.

Within the circle, there was a considerable area (vertically 4 inches, and horizontally over a radius of 4 inches) within which the source of radiation could be moved without significantly changing the recorded count more than 5 per cent. This error was no greater than that recorded by repeatedly counting an identical source without change of its position. Freedberg *et al.* (3) have reported similar observations. The multicounter was used to measure the amount of radiation emanating from a source of I^{131}. The source was either the thyroid gland of a subject or a vial containing radioactive iodine.

An aliquot of the ingested dose was placed in a vial and diluted to a volume of 10 ml. This was used as a standard source for the determination of the count without interference of any solid substance. This vial was placed in the center of the circle of the multicounter tubes. The count thus recorded, minus the background count in a given period of time, was designated the "100 per cent count." In those experiments in which radioactive iodine was ingested, the "thyroid uptake" and the "100 per cent count" were recorded twenty-four hours after the ingestion of the iodine. The subject's thyroid gland was centered in the circle between the four tubes, the count thus recorded being designated the "uptake count" of the thyroid gland. The percentage uptake of radioactive iodine by the thyroid gland was calculated by dividing the "uptake count" by the "100 per cent count" and multiplying this fraction by 100. In other experiments, the vial containing the I^{131} was strapped to the neck of subjects who had not previously ingested any radioactive isotopes. The subject was then seated so that the vial was in the center of the circle of the four G-M tubes. The count thus obtained was designated the "recorded count." The percentage recorded was calculated by dividing the "recorded count" by the "100 per cent count" and multiplying by 100.

Single-tube method. A high-efficiency gamma tube[3] was used as the single tube. The tube was shielded by a jacket of lead, $\frac{1}{2}$ inch in thickness. The unshielded end was placed exactly 35 cm. from the source of radiation (the anterior surface of the subject's neck or a vial containing I^{131}). The "100 per cent count," the "uptake count" of the thyroid gland, and the "recorded count" were determined, and the percentage uptake by the thyroid gland and the percentage recorded were calculated, as previously described.

All counts recorded in this paper were net counts (total count minus background count). Background counts were obtained by counting stray radiation by each method when there was no source of radioactivity in the counting room.

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[3] Sylvania Electric Products, Inc. of Boston—Model GG 306.

RESULTS

Comparative reliability of the two methods in recording I^{131} content

Counts were recorded of radiation from 10 patients' thyroids by each of the methods being studied. Each count was done in triplicate. The percentage error in count was considered to be the maximum difference between the three counts divided by the mean of these counts and multiplied by 100. Table 1 reveals that by the multitube method the mean error

TABLE 1. PERCENTAGE ERROR IN TRIPLICATE READINGS AS RECORDED BY SINGLE G–M TUBE AND MULTICOLOR METHODS

	Single-tube method %	Multitube method %
	1.8	6.0
	9.6	11.4
	0.0	2.1
	4.1	2.3
	0.3	8.7
	1.1	1.4
	0.4	1.9
	1.9	4.7
	2.3	5.3
	11.0	10.0
Mean	*3.3*	*5.4*

was 5.4 per cent compared to a mean error of 3.3 per cent by the single-tube method.

Comparative ability of the two methods in recording I^{131} content of a source

Each of 8 subjects ingested 100 microcuries of I^{131}. Twenty-four hours later, the percentage uptake by their thyroid glands was determined by the single-tube and by the multicounter methods. Table 2 presents these data. The thyroid uptake was 20 to 33 per cent greater as measured by the single-tube than by the multitube method.

The second experiment was planned to determine by which of these two methods the "recorded count" would more closely approximate the control "100 per cent count" from an unobstructed source of I^{131}. First the radiation from a vial containing I^{131} was counted. The vial was then strapped to a subject's neck and the count recorded by each method was noted. These data are presented in Table 3. The multicounter method

TABLE 2. PERCENTAGE UPTAKE OF I^{131} BY THYROID GLANDS AS RECORDED BY SINGLE G–M TUBE AND MULTITUBE METHODS

Single-tube method, % of "100% count"	Multitube method, % of "100% count"	% Loss with multitube method, % of single-tube "uptake count"
49.5	35.0	29
88.8	69.4	22
23.4	18.8	20
34.0	23.0	33
30.8	24.4	21
19.3	13.8	29
29.8	21.0	30
42.8	31.7	26

recorded 70 to 76 per cent of the control count, whereas the single-tube method recorded 105 to 110 per cent.

It had been suggested by Hertz (5) that the results in experiments 1 and 2 might be duplicated if the front and rear tubes of the multicounter were not of the same sensitivity. To evaluate this possibility the third experiment was performed as follows: Two patients who had ingested I^{131} sat within the circle of the multicounter tubes. The counts were first recorded as they faced the front tubes and then repeated as they faced the rear tubes. Table 4 reveals that there was no significant change in recorded counts, thus suggesting that the front and rear tubes were of similar sensitivity. To test the tubes further, a source of I^{131} was placed adjacent to each of the four tubes, as that tube was tested. There was less than a 5 per cent variation between the mean of these four counts and any one of these counts. These experiments tend to rule out unmatched tubes as the cause for the low counts recorded by the multitube method.

TABLE 3. PERCENTAGE RECOVERED FROM VIAL STRAPPED TO NECK AS RECORDED BY SINGLE G–M TUBE AND MULTITUBE METHODS

Single-tube method, % of "100% count"	Multitube method, % of "100% count"	% Loss with multitube method, % of "100% count"
105	76	24
110	70	30

Effect of passage of radiation through neck tissues upon recorded count

It was then postulated that the decreased "thyroid uptake" and "recorded counts" obtained by the multitube method, as compared with the single-tube method, might be due to absorption and scatter of radiation by the tissues of the neck. The fourth experiment was therefore planned to determine whether the passage of radiation through the tissue of the neck

TABLE 4. COMPARATIVE COUNTS, WITH THE SUBJECT FACING THE FRONT AND THEN THE REAR TUBE OF MULTICOUNTER

Facing front tube, counts/min.	Facing rear tube, counts/min.	Difference %
368	338	−8
325	337	+4

TABLE 5. PERCENTAGE RECOVERED FROM VIAL STRAPPED TO NECK AS RECORDED BY SINGLE G–M TUBE METHOD. EFFECT OF PASSAGE OF RADIATION THROUGH NECK

Neck behind source of radiation, % of "100% count"	Neck between source and G–M tube, % of "100% count"	% Lost by passage through neck, % of "100% count"
105	34	66
110	36	64

would decrease the count recorded by the single-tube method. Counts were obtained from a vial containing I^{131} kept at a constant distance of 35 cm. from the unshielded end of the G-M tube. In the first part of the experiment, the vial was in front of the subject's neck; in the second part, the neck was interposed between the source and the G-M tube. Table 5 presents the percentage recovered. About 65 per cent of the radiation from the vial failed to be recorded by the G-M tube when the neck of the subject was placed between the source of radiation and the vial.

Effect upon recorded count of slight variation of location of the source as the distance between the source of radiation and the G-M tube was increased

The final experiment was planned to determine the effect of the variation of 1 cm. in the location of the source upon the recorded count, as the distance between the source of radiation and the G-M tube increased. The distances between the source and the G-M tube were 1, 2, 3, 9, 10, 11, 19,

20, 21, 29, 30, 31, 39, 40 and 41 cm. In this experiment a G-M tube,[4] which was encased in a lead container $\frac{1}{2}$ inch thick with a side window measuring 1 by 5 inches, was used. The data presented in Table 6 demonstrate that as the distance between the G-M tube and the source of I^{131} increased, the

TABLE 6. EFFECT OF 1-CM. VARIATION OF LOCATION OF SOURCE OF RADIATION UPON COUNT, AS THE DISTANCE BETWEEN SOURCE AND G–M TUBE INCREASES

Distance between source and G–M tube (cm.)	Counts/min.	% Difference	Mean of % difference
1	17,000	42.5	
2	12,000		42.1
3	7,000	41.7	
9	2,220	13.3	
10	1,960		13.1
11	1,710	12.8	
19	759	7.8	
20	704		7.9
21	648	8.0	
29	361	3.1	
30	350		3.0
31	340	2.9	
39	216	6.4	
40	203		4.5
41	198	2.5	

variation of 1 cm. in the location of the source of radiation gave a diminishing variation in the recorded count. When these data were plotted on semi-log paper (Fig. 1), it became apparent that position of the source of radiation would cause less than a 5 per cent error if the distance between the edge of source of radiation and the side of the G-M tube was 30 cm. or more. These results were anticipated from the inverse-square law, *i.e.*, the intensity of radiation is inversely proportional to the square of the distance between the source and the point of measurement.

DISCUSSION

Multicounter method. From our data, it is obvious that with this multitube method of measuring radiation from the thyroid gland, there is a loss of 24 to 30 per cent of the radiation as compared with the control "100 per

[4] Radiation Counter Lab., Chicago, Illinois—Bismuth Wall Gamma Counter, Mark 1, Model 12.

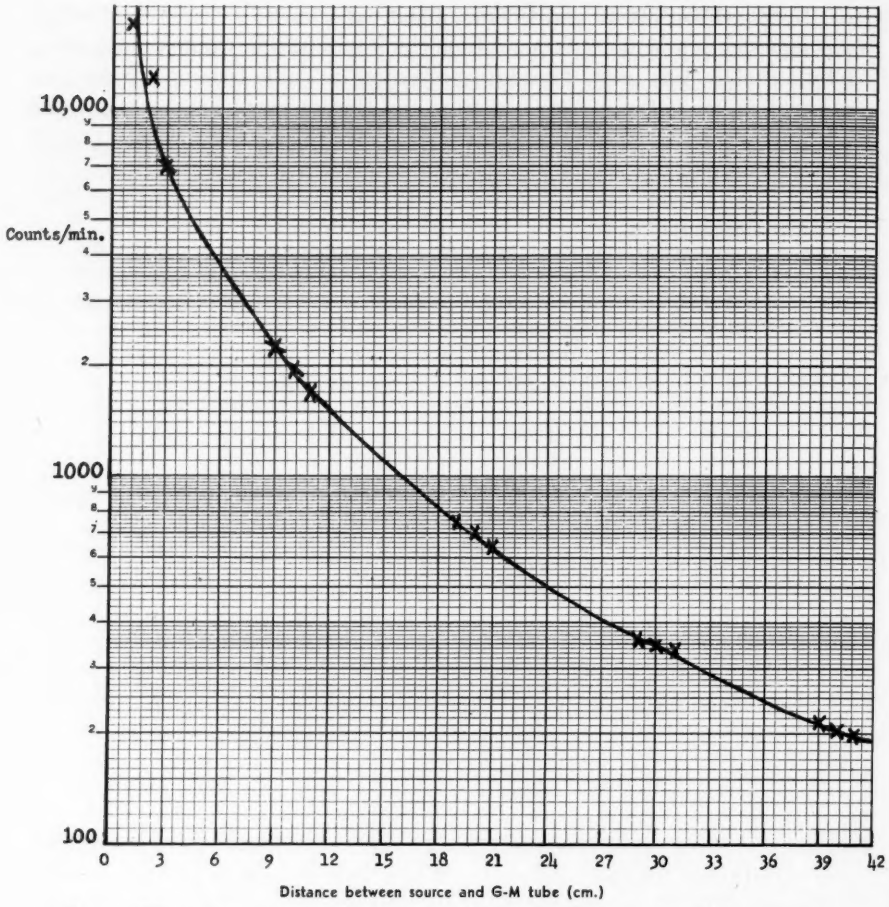

FIG. 1. Effect of 1-centimeter variation of location of source of radiation upon count, as the distance between source and G-M tube increases.

cent count." Our data also indicate that the reason for this is absorption or scatter of some of the radiation as it traverses the tissue of the neck. However, we are not in agreement with Freedberg *et al.* (3) who reported that the radiation recorded by the four G-M tube multicounter, from the I^{131} within a vial, was not decreased by surrounding the vial with meat and bones (thus simulating a neck). They also observed that there was no decrease in recorded radiation if the vial was strapped to the neck of a subject. We cannot explain the discrepancy between their data and our findings unless the characteristics of their G-M tubes differed from those present in the multicounter which we tested.

It is of interest that the measured error of the multitube method as shown in Table 3 can be calculated from the results of the fourth experiment. Utilizing the data that $\frac{2}{3}$ of the control count was lost by the passage of radiation through the neck and that there was a gain of 8 per cent by "back scatter" (when the neck was behind the source of radiation), the expected loss is as follows: In the balanced four-tube counter, each tube registered $\frac{1}{4}$ of the total count when the source of radiation was in the center of the circle formed by the four tubes. However, when the vial containing I^{131} was strapped to the neck of a subject, each of the two rear tubes would fail to record $\frac{2}{3}$ of the count. In other words, $\frac{2}{3}$ of half the count, or $\frac{1}{3}$ of the total count, would be lost. The two front tubes would record an increment of 8 per cent each, or there would be a gain of 4 per cent of the total count. The summation of the 33 per cent loss and the 4 per cent gain would be an overall loss of 29 per cent. This is in close agreement with the observed losses of 24 and 30 per cent (Table 3) as measured by the multicounter.

An advantage of the multicounter technique is that variations in location and size of the thyroid gland cause no significant error.

Single-tube method. On the other hand, the single-tube method gives results which are 5 to 10 per cent greater than the control "100 per cent count." This is probably due to "back scatter" as described by Myant *et al.* (6) in a similar experiment. They reported that there was an increment of count if a source of radiation was placed in front of a solid object such as the neck. They explained this increment as due to "back scatter," *i.e.*, the radiation, which travelled from the source towards the neck, struck the solid neck and some rays were scattered in the direction of the G-M tube and were registered there. This accounted for the increment over the control count.

Error due to size and location of the gland may be appreciable with this technique, but our results demonstrate that if the G-M tube and source are at least 30 cm. apart, this error is small.

Thus both techniques have inherent flaws, some of which cannot be corrected or overlooked. The error of the multitube counter is large and is probably due primarily to absorption and scatter of radiation through neck tissue interposed between the thyroid gland and the two rear tubes. The error of the single-tube method due to "back scatter" is much smaller. The previous objection to the single G-M tube method, *i.e.*, error due to variation in location or size of the thyroid, can be overcome to a large extent by placing the G-M tube at least 30 cm. from the neck. Very sensitive G-M tubes, which have recently become available, will have to be used in order to keep the tracer dose of I^{131} to a minimum. We have been able to get satisfactory clinical results with tracer doses of 20 microcuries of I^{131}.

SUMMARY AND CONCLUSIONS

1. The efficacy of a single G-M tube and a four-tube multicounter method in assaying the I^{131} content of the human thyroid gland have been compared.

2. The mean error of triplicate readings by the single-tube method was 3.3 per cent compared to a mean error of 5.4 per cent by the multitube method.

3. As recorded by the single-tube method, the thyroid gland uptake was 20 to 33 per cent greater than the uptake recorded by the multicounter method.

4. When the source of radiation was in a vial containing I^{131} and was strapped to the neck, the single-tube method recorded an increment of 5 to 10 per cent, whereas the multicounter method recorded a decrement of 24 to 30 per cent. When the neck was interposed between the source and the single G-M tube, only 35 per cent of the control count was recorded.

5. Despite a 1-cm. variation in the position of the source of radiation, the single-tube method recorded counts with an error of less than 5 per cent when the G-M tube was at least 30 cm. from the source.

6. It is concluded that the single G-M tube method is satisfactory, provided that sensitive gamma-counting tubes are used and provided that the distance between the G-M tube and the patient's neck is not less than 30 cm.

7. According to our data, the single G-M tube method, if used as above described, is preferable to the multitube method used in these experiments.

Acknowledgment

The authors are indebted to the late Dr. Saul Hertz and to Mr. L. W. Cronkhite of the Atomic Instrument Co. for the use of their multicounter. They wish to express their gratitude to Dr. Robert Loevinger for his aid in interpreting the data.

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THE LEVELS OF 17-HYDROXYCORTICOSTEROIDS IN PERIPHERAL BLOOD OF HUMAN SUBJECTS*†

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BY USE of the method of blood analysis for 17-hydroxycorticosteroids described by Nelson and Samuels (1) it has been possible to measure levels of these corticosteroids in the peripheral arterial and venous blood of a number of human subjects.

Although adrenal responsiveness has been estimated by such techniques as eosinophil counts and urinary excretion of steroids, no previous direct measurements of the levels of cortical hormone in peripheral blood have given quantitative results. The biologic assay methods (2, 3) which have been applied either lack sufficient sensitivity or have large probable errors. The chemical methods used by others (4) have been nonspecific, resulting in considerable overestimation when applied to the crude extracts which have been used.

EXPERIMENTAL

Samples of approximately 30 ml. of blood were drawn and immediately placed in small bottles containing 3 mg. of heparin. Samples were refrigerated and the steroid determinations were run the same or the following day whenever possible. Occasional samples were held as long as three days. To check the stability of the compound, samples of the same blood were analyzed at intervals over a period of seven days. No significant variation in the results of the analysis occurred. It seems highly improbable, therefore, that any error could have been introduced because of the time during which any sample was held.

The method of determination of 17-hydroxycorticosteroids was that described by Nelson and Samuels (1). Chloroform-ether extracts of blood were partitioned between 70 per cent ethanol and hexane (Skellysolve B), chromatographed on magnesium silicate, and the fraction containing the

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‡ Public Health Service postdoctoral fellow, 1950–51.

17-hydroxycorticosteroids analyzed by a modification of the method of Porter and Silber (5).

When possible, arterial and venous samples were drawn simultaneously. The arterial blood was generally taken from the femoral artery and the venous blood from the antecubital vein. When an inflammatory condition was present, samples of arterial and venous blood were drawn across the area of active involvement.

The levels of 17-hydroxycorticosteroids in the peripheral blood of normal medical and graduate students have varied between 4 and 10 gamma per 100 ml. of whole blood. Significant arterio-venous differences have not been

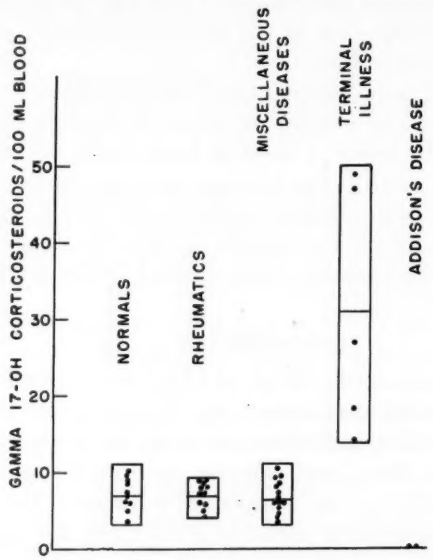

FIGURE 1

found in normal limbs or in blood obtained from across active rheumatoid joints. Concentrations in the peripheral blood of patients with acute rheumatic fever or rheumatoid arthritis have not showed significant variation from normal (Fig. 1).

Three patients with Addison's disease had no 17-hydroxycorticosteroids in their blood.

By the use of the paper chromatographic method described by Burton, Zaffaroni, and Keutmann (6) the compound measured has been demonstrated to move with 17-hydroxycorticosterone (Compound F.) No evidence for the presence of 17-hydroxy 11-dehydrocorticosterone (Compound E) was found, but the possibility that a smaller amount of 17-

hydroxy 11-desoxycorticosterone (Compound S) is present has not been ruled out.

A typical result following the administration of a single intravenous dose of 15 mg. of ACTH is demonstrated in Figure 2. The intravenous administration of ACTH has resulted in every case in an immediate increase in 17-hydroxycorticosteroid levels, the peak levels being obtained usually at about one hour after injection.

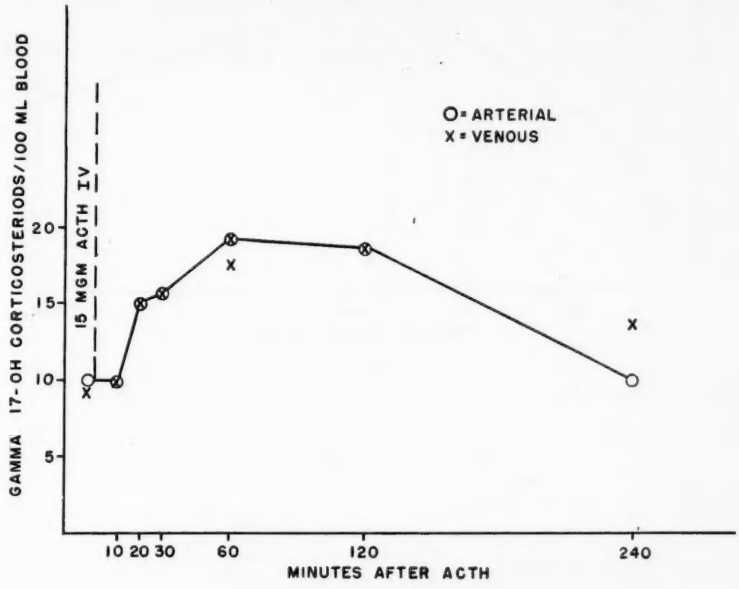

FIGURE 2

When the ACTH was administered intramuscularly the blood steroid level rose more slowly, reaching a peak about two hours after the injection (Fig. 3). The level had returned toward, but not to normal, four hours after the ACTH was given. Several values obtained six hours following intramuscular ACTH administration have demonstrated normal blood levels.

When the ACTH was given by continuous intravenous drip over a period of three hours, the blood level of 17-hydroxycorticosteroids rose steadily throughout the period of infusion (Fig. 4). Smaller doses of ACTH given in continuous intravenous infusions over a period of twenty-four hours have resulted in much higher levels of corticosteroids than could be obtained by any other means of administration (Figs. 5 and 6).

A study was undertaken to determine the relative effectiveness of intra-

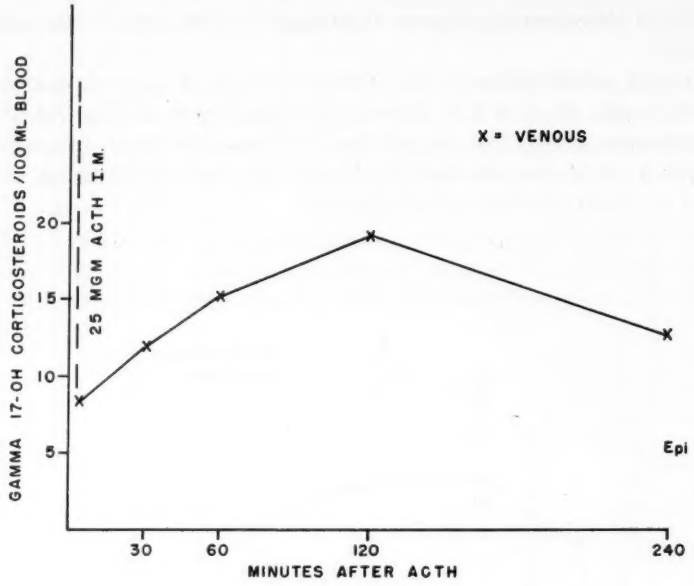

FIGURE 3

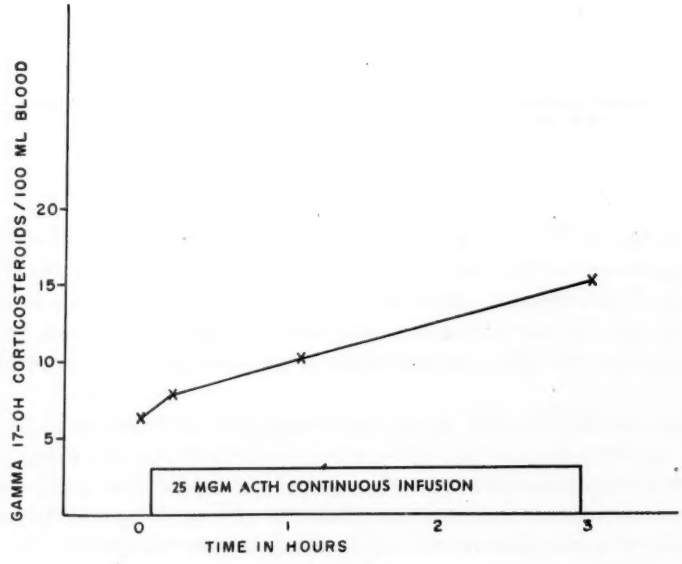

FIGURE 4

muscular, subcutaneous, and continuous intravenous infusion of ACTH in maintaining increased levels of 17-hydroxycorticosteroids and to determine whether there was a correlation of blood levels with clinical responsiveness to ACTH. The first subject was a 40-year-old white male who had active rheumatoid arthritis with a number of hot, red, swollen joints. The results are illustrated in Figure 5. Arterial and venous samples were

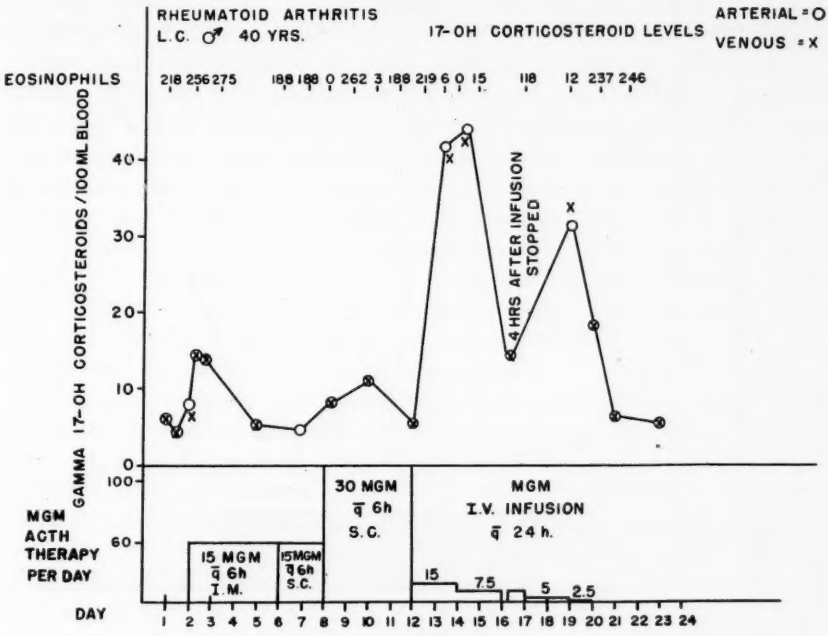
RHEUMATOID ARTHRITIS
L.C. ♂ 40 YRS.
17-OH CORTICOSTEROID LEVELS
ARTERIAL = O
VENOUS = X
EOSINOPHILS
218 256 275 188 188 0 262 3 188 219 6 0 15 118 12 237 246
GAMMA 17-OH CORTICOSTEROIDS / 100 ML BLOOD
40
30
20
10
0
4 HRS AFTER INFUSION STOPPED
MGM ACTH THERAPY PER DAY
100
60
15 MGM q 6h I.M.
15 MGM q 6h S.C.
30 MGM q 6h S.C.
MGM I.V. INFUSION q 24 h.
15
7.5
5
2.5
DAY
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

FIGURE 5

drawn throughout the experiment. No significant arterio-venous differences could be demonstrated. Four hours after the end of an infusion the blood level had fallen almost to normal. Restarting of the infusion with only 5 mg. per twenty-four hours brought the corticosteroid concentration up again to high levels. The infusion of 2.5 mg. per twenty-four hours was sufficient to maintain fairly high blood levels.

This patient's clinical response to therapy closely paralleled the changes in the blood levels of adrenal steroid. Although some improvement in the arthritic condition was observed while ACTH was being administered intramuscularly or subcutaneously, much more remarkable improvement occurred shortly after intravenous therapy was instituted.

The course of a second patient also given intramuscular, subcutaneous, and intravenous infusions of ACTH is demonstrated in Figure 6. This patient was a 32-year-old white female who had acute lupus erythematosus disseminatus. Again much higher levels of blood cortical steroid were found when the ACTH was given by intravenous infusion.

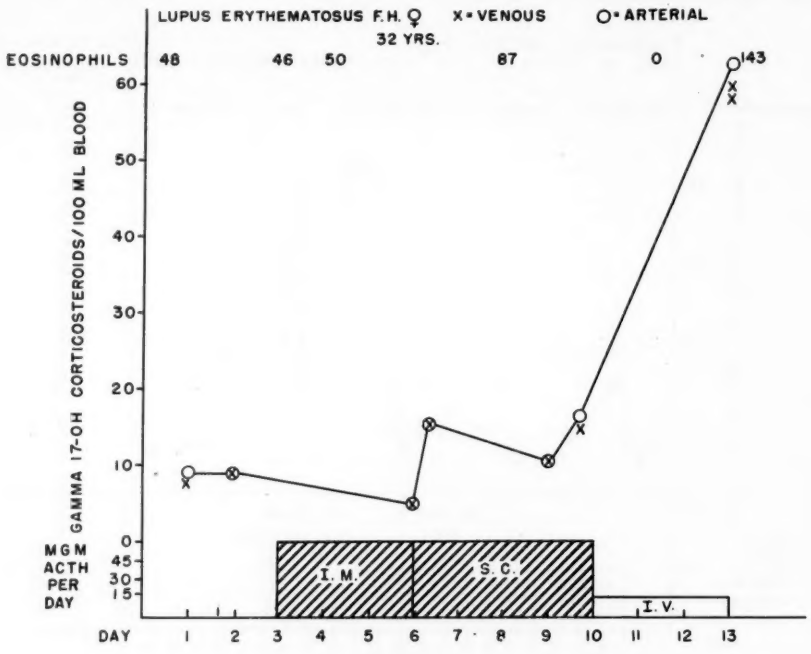

FIGURE 6

A third case, in which the intravenous administration of ACTH was followed by a series of intramuscular injections, also demonstrated the much greater effectiveness of the continuous infusion method.

DISCUSSION

The failure to find concentrations of 17-hydroxycorticosteroids outside the normal range in the blood of chronically or acutely ill patients, except when the patient was in a terminal condition, would indicate that adrenal steroid production was in balance with removal.

Since there was no measurable increase in the removal of these corticosteroids when blood circulated through areas of inflammatory involvement, it seems that any increase in the rate of release of ACTH in these condi-

tions must have been less than the amount of ACTH which was given to one of our subjects by continuous intravenous drip for three hours. In the latter instance there was a definite and continuous increase in the concentration of these steroids in the blood to levels which soon exceeded the normal range. When, however, the human being is *in extremis* the stimulus for ACTH release is such as to produce levels of steroid in the blood similar to those found during therapy by intravenous drip of 15 mg. of ACTH over a period of twenty-four hours.

That the material measured in the blood at this time is physiologically active is indicated by the history of the first patient in which this terminal rise was observed. This patient was an elderly woman who was recovering from bronchopneumonia and receiving antibiotic therapy. Although her pneumonia was clearing and she was afebrile, hyperleucocytosis, hypokaliemia, and the picture of metabolic alkalosis developed. The similarity of her clinical picture to that of hyperadrenocorticism was noted and a sample of blood was therefore drawn to be analyzed for 17-hydroxycorticoids. The level was found to be 48 gamma per 100 ml. It would seem from this history that the excessive response of the adrenal in severe disease may in itself sometimes be detrimental.

These studies for the first time establish the great lability of the pituitary-adrenal system. Within less than fifteen minutes after completing the injection of ACTH into the circulation, the level of 17-hydroxycorticosteroids in the blood began to rise. The same prompt response followed electroshock therapy. When intravenous infusion of the pituitary hormone had been maintained at a slow but steady rate for some time, cessation of the infusion led to a rapid drop in the concentration of 17-hydroxycorticosteroids in the blood. To achieve such rapid adjustments both pituitary and adrenal hormones must be rapidly released and removed from the circulation. Sayers and coworkers (7) demonstrated that metabolic changes occurred in the adrenal glands of the rat or guinea pig immediately after the injection of ACTH, and Sayers *et al.* (8) found a marked increase in the excretion of 17-ketosteroids and reducing steroids after the injection of large doses of ACTH intravenously into human subjects. The results here show that these phenomena are associated with the immediate secretion of the chemical compounds known to have adrenal hormonal action. Further, they (8) found that the pituitary hormone disappeared rapidly from both the blood and tissues (9).

Associating the results of Sayers and coworkers in humans with ours, it seems that there is a significant time factor involved in each of the physiologic processes, and thus a sequence of events. When single large doses of ACTH were given intravenously in our studies the levels of cortical

steroids reached a maximum at about one hour after injection and had returned to normal levels by three hours. On the other hand, the data of Sayers *et al.* would indicate that adrenal secretion, indirectly measured as the fall in eosinophils and the excretion of 17-ketosteroids, reached a maximum about three hours after the injection. Changes in electrolytes became most marked in the 6- to 12-hour period. The ultimate action of the hormones appears to involve metabolic systems which require time for maximum adjustment.

The much greater effectiveness of ACTH given by continuous infusion has previously been observed by Ingle (10), Gordon (11), and Thorn (12). The results of this study indicate that this increased effectiveness is specifically due to the much higher levels of adrenal steroids which are maintained in the general circulation when a given amount of ACTH is administered in this way. These findings indicate that excessive amounts of the pituitary hormone given over short periods of time are disposed of without exerting a proportionate action on the adrenals.

Although the failure to observe a measurable difference between arterial and venous levels of the cortical steroids, even across severely inflamed areas, argues against a rapid removal in normal or damaged peripheral tissues, changes in levels of as much as 10 to 20 per cent might have occurred without being conclusively identified. Since total flow per unit of time was large in any system across which such samples were taken, considerable amounts could have been removed without causing consistently measurable changes. If, however, this were a normal process the arterio-venous differences in as large a series as observed here should have shown a significant positive trend. This was not observed, and it seems probable, therefore, that normal peripheral tissues do not ordinarily remove any great amount of cortical steroids from the blood.

SUMMARY

The levels of 17-hydroxycorticosteroids in peripheral blood have been determined in normal subjects and in a number of clinical conditions. Concentrations in the normal subjects were between 4 and 10 gamma per 100 ml. of blood. Values in the various diseased patients were within the normal range, except when a fatal outcome was imminent; they were then markedly increased.

Simultaneous arterial and venous samples were drawn in most instances. There was no consistent trend in the arterio-venous differences, which were in nearly all cases within the limits of error of the method. This was true even across areas of severe inflammation. It would seem that the peripheral tissues do not remove the cortical steroids rapidly from the blood.

Treatment with adrenocorticotropic hormone led to an increase in concentration of the 17-hydroxycorticosteroids. With a single intravenous injection, the level was maximal at approximately one hour and returned to the pre-injection value within three hours. When an intramuscular injection was given, the maximal level was reached in about two hours and the concentration fell again to normal in four to six hours. Continuous intravenous drip caused a slow, steady rise in the concentration of the steroids to levels which, at the end of twenty-four hours, were higher than those achieved by any other method; the total dose required was also much smaller. The concentration fell rapidly on cessation of the intravenous drip. The lability of the pituitary-adrenal system indicates that the pituitary and adrenal hormones are both rapidly released and rapidly disposed of.

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Letter to the Editor

MODIFICATION OF THE CLINICAL AND METABOLIC EFFECTS OF ACTH BY DIETARY MEANS*

To the Editor

During the long-term administration of ACTH to patients with chronic diseases, it has been found that:

1. In a significant number of patients untoward effects develop, which are of sufficient magnitude to make continuation of therapy difficult or impossible.

2. A certain number of patients become resistant to the therapeutic effects of ACTH in a relative or absolute sense.

Many observers have noted that limitation of sodium intake decreases the tendency to ACTH-induced and cortisone-induced edema and hypertension and that potassium supplementation appears to diminish untoward effects. In this clinic it has been found that the limitation of sodium to a level below 300 mg. per day is effective in preventing edema formation in the majority of patients. Even with such sodium limitation and potassium supplementation, however, a significant number of individuals during long continued therapy show "toxic" effects which, in a relative sense at least, exceed therapeutic effects.

One such individual was a young male with rheumatoid arthritis who had been under study on the metabolic ward for a period of five months. During the first several weeks of treatment, he made an excellent clinical response to intermittent ACTH and cortisone therapy. By the fifth month, however, despite limitation of sodium intake, dietary supplementation with potassium, and a trial of ACTH derived from beef pituitary (to determine whether the resistance to ACTH might be on an antibody basis), the status of the patient's joints was deteriorating rather than improving, and his sedimentation rate had risen to relatively high levels. Severe acne, marked rounding of the face, edema, weakness, and marked despondency had also developed.

As part of a program of evaluation of effects of ACTH and adrenal steroids upon fat metabolism, he was given a formula diet containing only

* This work was supported in part by a grant from the Armour Laboratories (Research and Medical Divisions), Chicago, Illinois.

fat, equivalent to 1,950 calories daily[1], plus standard salt and vitamin supplementation (Fig. 1). After three days, protein was added to the

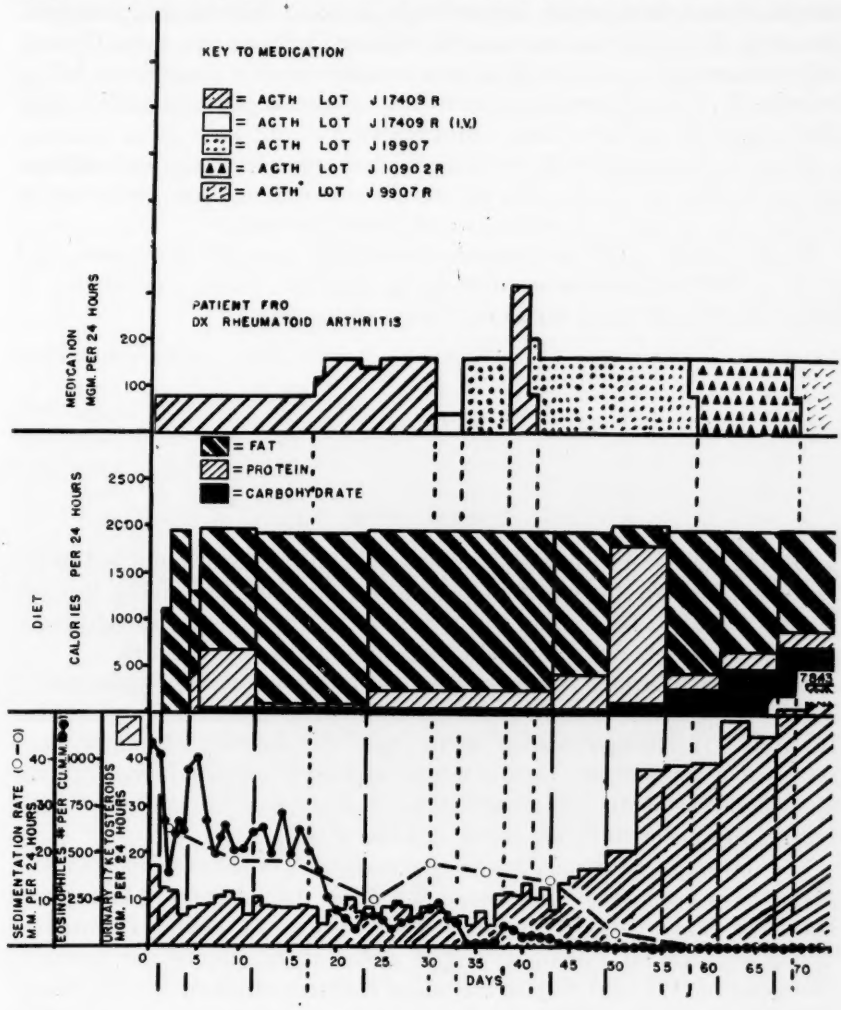

FIG. 1. *Dietary modification of ACTH effects.* The change in 17-ketosteroid excretion would be compatible with alteration of metabolism of adrenal steroids.

diet. Seven days later the essentially "pure fat" diet was resumed and continued for twelve days. Six days after the resumption of this diet,

[1] The fat diet consisted of emulsified salad oil. Protein was derived entirely from calcium caseinate (Casec).

the "mooning" of the face had virtually disappeared; there was no edema; acne had diminished; the patient's energy and euphoria had greatly increased; and considerable improvement in joint function had occurred. The dose of ACTH was increased to 160 mg. daily at this time. Clinical improvement progressed rapidly in association with a progressive fall in eosinophils and sedimentation rate. No untoward effects resulted from this large dose, and acne slowly disappeared.

It seemed probable that the described changes were not coincidental and were related in some way to the diet. It was decided, therefore, to embark on a program of evaluation of dietary factors.

With calories, salts, and vitamins remaining constant throughout, and with ACTH administration remaining constant (except as shown in Figure 1), the following dietary program was pursued:

Diet period	*No. of days*	*Protein (Gm.)*	*Fat (Gm.)*	*Carbohydrate (Gm.)*
4	20	43.3	190.2	13.0
5	6	103.7	162.5	13.0
6	6	425.0	24.5	26.0
7	6	43.3	170.4	63.0
8	6	43.3	143.4	123.0
9	6	43.5	118.4	180.0

During periods 4 through 7 the patient's clinical status remained essentially unchanged. Increased "mooning" of the face was noted through periods 8 and 9. Before the end of period 9, the patient had significant increase in acne and complained of weakness and some dizziness.

From the foregoing, it appeared that increased carbohydrate intake and/or decreased fat intake accentuated the toxic manifestations resulting from ACTH administration; or, to state the matter differently, high fat, with or without high protein intake, appeared compatible with optimal clinical effects of ACTH. Observations in other patients currently under treatment appear to be in accord with the above.

17-ketosteroid excretion. Prior to the institution of the fat diet, and with the same or nearly the same dosage of ACTH, the patient's 17-ketosteroid excretion had averaged approximately 18 mg./24 hours. During the period of high fat intake it will be noted that, despite the increased dosage of ACTH, and despite the major evidence of adrenocortical stimulation (clinical improvement, fall in sedimentation rate, and fall in eosinophils to zero) the 17-ketosteroid excretion remained for the most part below 10 mg./24 hours until the protein and subsequently the carbohydrate were increased in the diet, at which time the 17-ketosteroid excretion rose to values in excess of 70 mg./24 hours. We have recently observed a similar phenomenon in another patient who received as much as 320 mg. of ACTH daily during a period of pure fat intake. In this patient also, no

evidence of "toxic" ACTH effects were observed until the patient had returned to a general diet.

The explanation for the change in 17-ketosteroid excretion must for the present be speculative. It is conceivable that exclusive or nearly exclusive fat intake in some way modifies the metabolism of steroid hormones. The change in clinical response to ACTH administration might be related partially to such modification of steroid metabolism. This thesis does not explain the continued optimal clinical effect, in conjunction with rising 17-ketosteroid excretion, occurring during the periods of protein supplementation.

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Acknowledgment

Grateful acknowledgment is made to Dr. John Mote of the Armour Laboratories for supplies of ACTH; to Dr. Warren Cox of Mead Johnson & Company for supplies of "Casec," and to Mr. C. D. Pratt of Atlas Powder Company for supplies of "Tween," the emulsifying agent used.

† Schering Research Fellow in Endocrinology, 1950–51.
** Damon Runyon Clinical Research Fellow, 1949–51.

The Association for the Study of Internal Secretions

THE 1952 ANNUAL MEETING

The Thirty-fourth Annual Meeting of The Association for the Study of Internal Secretions will be held at the Palmer House, Chicago, Illinois, Thursday, Friday, and Saturday, June 5–6–7, 1952.

The Local Chairman of Arrangements is Dr. Willard O. Thompson.

All Scientific Sessions will be held in the Palmer House. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, June 7, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

All members are urged to make hotel reservations immediately, inasmuch as the hotels expect to be filled to capacity. Make reservations directly with the Palmer House, advising time of arrival and departure date. Make your reservations now and avoid disappointment.

Those wishing to present papers, which will be limited to ten minutes, should send four copies of the title and abstract to the Vice-President, Dr. Evelyn Anderson, National Institutes of Health, Bethesda 14, Maryland, not later than *March 1, 1952. It is imperative that the abstracts be informative and complete with results and conclusions in order that they may be of reference value and suitable for printing in the program and journals of the Association.* The following regulations for the preparation of abstracts and titles must be carefully followed to insure consideration of the paper for the program:

1. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. References, if used, must be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.
2. The title heading must be arranged as follows:
   Line 1. Title, not to exceed fifteen words.
   Line 2. Author/s. The name of each non-member-author collaborating with member-authors is to be followed by the phrase

"(by invitation)." Names of non-members who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be given after his name.

Line 3. Institution of origin and city in which institution is located.

3. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three copies.
4. Abstracts should be letter-perfect, since there will be no opportunity for proofreading by authors.

RECIPIENTS OF AWARDS FOR 1951

THE SCHERING FELLOWSHIP

The Schering Fellowship for 1951 was awarded to *Dr. John Duane Stoekle,* to permit him to study the factors controlling the peripheral use of thyroid hormone and related problems, under the direction of Drs. J. H. Means and John B. Stanbury.

Dr. Stoekle received his B.S. degree at Antioch College in 1944 and his M.D. degree in 1948 at Harvard. Since his graduation he has served as interne and medical resident at the Massachusetts General Hospital.

THE SQUIBB AWARD

The Squibb Award for distinguished contributions to endocrinology was awarded in 1951 to *Dr. James Bertram Collip.*

Dr. Collip was born in Belleville, Ontario. After receiving his doctorate in biochemistry from the University of Toronto in 1916, he spent the next five years as a member of the department of biochemistry at the University of Alberta. Following the isolation of insulin by Banting and Best in 1921, he returned to the University of Toronto and joined the group then engaged in the intensive studies on the isolation and biologic properties of this hormone. His contributions to this group study are too well known to require recapitulation at this time.

He then returned to Alberta as head of the department of biochemistry and almost at once, there came from his laboratory a series of brilliant papers on the isolation and properties of the parathyroid hormone, as well as further significant contributions on the mechanism of the action of insulin and on carbohydrate metabolism.

In 1928 he left the West to become Professor of Biochemistry at McGill, succeeding his beloved mentor, the late A. B. McCallum. The work of his pupils and himself during the next thirteen years, on the hormones of the placenta and anterior pituitary, is now one of the classics of endocrinology. The demonstration of the existence of conjugated estrogens in the placenta, the development of methods for the separation of anterior pituitary hormones, the concept of antihormones, to say nothing of papers on many other phases of endocrinology and biochemistry, appeared in rapid succession from his department.

When Canada entered the war in 1939 Dr. Collip not only assumed large responsibilities in the National Research Council of Canada, but directed the important studies on shock that were carried on in his department. His contributions to the war efforts of his country were recognized by his appointment in 1943 as Commander of the Order of the British Empire.

In 1947 he left McGill to become Dean and Professor of Medical Research at The University of Western Ontario, positions he holds at the present time.

THE CIBA AWARD

The Ciba Award for 1951 was given to *Dr. Albert Segaloff.*

Dr. Segaloff's earlier studies have provided a thorough survey and analysis of the effects of thymus extracts, thymocrescin, and thymectomy in successive generations of rats. He has contributed materially to our understanding of the effect of the liver upon various estrogenic and related steroids through his studies comparing the physiologic activities of these substances when administered intrasplenically and subcutaneously. His studies in the field of experimental carcinogenesis in the rat and mouse have contributed materially to this area of cancer research. In more recent years, although he has maintained his interest and activity in studies upon laboratory animals, he has also extended and applied his investigative abilities to numerous problems in clinical endocrinology.

Dr. Segaloff was born in Connecticut in 1916. He received the B.S. degree from Yale University in 1937, the M.S. degree in Anatomy in 1939 from Wayne University and the M.D. degree from the same institution in 1943. Since 1945 he has been a member of the staff and director of Endocrine Research of the Alton Ochsner Medical Foundation, New Orleans, Louisiana.

1952 AWARDS AND FELLOWSHIPS

Fellowships

THE SCHERING FELLOWSHIP IN ENDOCRINOLOGY

This Fellowship will not be awarded in 1952, so that it may accumulate toward a $5,000 Fellowship to be awarded in 1953.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

The Ayerst, McKenna and Harrison Fellowship was first awarded in 1947 to Dr. Samuel Dvoskin and in 1948 to Dr. Ernest M. Brown, Jr. Dr. Brown was re-elected for the Fellowship in 1949. The 1950 Award was made to Dr. Lawrence E. Shulman.

Association Fellowships are designed to assist men or women of exceptional promise in their progress toward a scientific career in endocrinology. Fellowships may be awarded to an individual who possesses the Ph.D. or M.D. degree or to a candidate for either of these degrees. The stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointee. The Committee will, in reviewing the proposed program of study, consider the amount of time which the Fellow intends to spend in course work and/or teaching. He must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals. He must submit a program of proposed study, indicate one or more institutions where the proposed program will be followed, and submit statements of approval from the investigators with whom he proposes to conduct his research. He must serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

Awards

THE E. R. SQUIBB AND SONS AWARD

The E. R. Squibb & Sons Award of $1,000 was established in 1939, and was given first in 1940 to Dr. George W. Corner; in 1941 to Dr. Philip E. Smith; in 1942 to Dr. Fred C. Koch; in 1944 to Dr. E. A. Doisy; in 1945 to Dr. E. C. Kendall; in 1946 to Dr. Carl G. Hartman; in 1947 to Drs. Carl F. and Gerty T. Cori; in 1948 to Dr. Fuller Albright; in 1949 to Dr. Herbert M. Evans; in 1950 to Dr. C. N. H. Long; and in 1951 to Dr. J. B. Collip. In 1943 no award was given. A special committee of five members of the Association selects the recipient from among investigators in the United States or Canada, on the basis of outstanding contributions to endocrinology.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishment of an investigator not more than thirty-five years of age in the field of clinical or pre-clinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the award was presented to Dr. E. B. Astwood; in 1945 to Dr. Jane A. Russell; in 1946 to Dr. Martin M. Hoffman; in 1947 to Dr. Choh Hao Li; in 1948 to Dr. Carl Heller; in 1949 to Dr. George Sayers; in 1950 to Dr. Oscar M. Hechter; and in 1951 to Dr. Albert Segaloff. The Award is for $1,200. If, within twenty-four months of the date of the Award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $1,800.

Nominations

Each member has the privilege of making one nomination for each Fellowship or Award. A nomination should be accompanied by a statement of the importance of the nominee's contributions to, or interest in, endocrinology and by a bibliography of the nominee's most important publications, with reprints if possible. The nominations should be made on special application forms which may be obtained from the Secretary, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City 3, Oklahoma, and returned to him *not later than March 1, 1952.*

ENDOCRINOLOGY INDEX

The Association for the Study of Internal Secretions has undertaken to prepare a comprehensive Index of the articles published in *Endocrinology*. This Index will appear in three volumes. Volume III, covering papers published in 1940 through June, 1947, in volumes 26–40 of the journal, has been completed and is now available for distribution. Volumes I and II of the Index have not yet been completed.

Volume III of the Index is available gratis to members of the Association for the Study of Internal Secretions. The price to all non-members is $2.50. Inquiries and orders should be addressed to:

Dr. H. H. Turner, Secretary
1200 North Walker Street
Oklahoma City 3, Oklahoma.

The American Goiter Association

THE 1952 ANNUAL MEETING

The next Annual Meeting of the American Goiter Association will be held in St. Louis, Missouri, May 1, 2 and 3, 1952, at the Hotel Chase. All those who plan to attend are urged to make hotel reservations immediately.

VAN METER PRIZE AWARD FOR 1952

The American Goiter Association again offers the Van Meter Prize Award of three hundred dollars and two Honorable Mentions for the best essays submitted concerning original work on problems related to the thyroid gland. Providing essays of sufficient merit are presented in competition, the Award will be made at the annual meeting of the Association which will be held in Saint Louis, Missouri, May 1, 2 and 3, 1952.

The competing essays may cover either clinical or research investigations; should not exceed three thousand words in length; must be presented in English; and a typewritten double-spaced copy in duplicate sent to the Corresponding Secretary, Dr. George C. Shivers, 100 East Saint Vrain Street, Colorado Springs, Colorado, *not later than March 1, 1952*. The committee, who will review the manuscripts, is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for presentation of the Prize Award Essay by the author, if it is possible for him to attend. The essay will be published in the annual Transactions of the Association.

ARMOUR'S INTERNATIONAL SYMPOSIUM ON CHEMISTRY OF ACTH

Armour and Company will sponsor an international symposium in the Palmer House, Chicago, September 18, 1951, at which leading European and American biochemists will discuss the chemistry of ACTH.

The conference will follow within a few days the Jubilee meeting celebrating the 75th birthday of the American Chemical Society, together with the meetings of the International Chemical Congress and the International Chemical Union, in New York and Washington.

Many prominent foreign chemists will be in the United States for these sessions, most of them as guests of American chemical and pharmaceutical organizations. Armour research division, headed by Victor Conquest, vice-president, has interested itself especially in three younger men, two English and one Swedish, who have never before had an opportunity to meet American biochemists.

These three are: George W. Kenner, chemist, and Fred Sanger, biochemist, both of Cambridge University, England; and Jerker Porath, biochemist of Uppsala University, Sweden.

Topflight visitors will also include: Prof. Arne Tiselius, biochemist of the University of Uppsala, Sweden, winner of the Nobel prize in chemistry in 1948; Prof. A. R. Todd, Cambridge biochemist; and C. J. O'R. Morris of the London, England, County Hospital.

Scheduled to present formal papers are: Prof. David F. Waugh, biochemist of the Massachusetts Institute of Technology; E. B. Astwood, M.D., Tufts College, biochemist; Emil M. Smith, M.D., and Prof. George W. Sayers, biochemist, both of the University of Utah; Prof. Sidney W. Fox, biochemist, Iowa State University; Prof. Wendell M. Stanley, C. H. Li and J. Ieuan Harris, biochemists, and Fred W. Carpenter, virologist, all of the University of California.

Wilfred White and Joseph D. Fischer, biochemists of the Armour and Company research division, will also deliver papers.

"One of the most important chemical frontiers is the structure of proteins," Mr. Conquest explained. "We feel certain that bringing together a group of men so outstanding in that exploration as these men are for exchange of ideas and discussion will advance the whole process tremendously."

American Association for the Advancement of Science

PHILADELPHIA MEETING, DECEMBER 26–31, 1951

The 118th Meeting of the American Association for the Advancement of Science, the annual meeting for 1951, will include programs of all 18 of the Association's sections and about 45 participating societies. Focus of the approximately 225 sessions will be Convention Hall, adjacent to the University of Pennsylvania's School of Medicine and its associated hospitals; there will be some meetings in the downtown hotels, especially the Bellevue-Stratford (headquarters) and the Benjamin Franklin (zoologists). Programs of particular interest to those in medicine and experimental biology include:

SECTIONAL PROGRAMS

AAAS Section C-Chemistry:

Eleven sessions, including one on Medicinal Chemistry, *December 28;* two on Forensic Sciences *December 29;* and two on Reaction Mechanisms, *December 26, 27.*

AAAS Section F-Zoological Sciences:

A symposium, Estuarine Ecology, jointly sponsored by the American Society of Limnology and Oceanography, American Society of Zoologists, and the Ecological Society of America, *December 30;* a two-session symposium, Sex in Microorganisms, jointly sponsored by AAAS Section G, the American Society of Protozoologists, American Society of Zoologists, and the Botanical Society of America, *December 30.*

AAAS Subsection Nm-Medicine:

Four-session symposium on Abnormalities of Lipid Metabolism, with special reference to arteriosclerosis, *December 29, 30;* plus a medical scientists' dinner, *December 29,* at which Malcolm Soule will deliver a vice presidential address.

AAAS Subsection Nd-Dentistry:

Three sessions, including a symposium on Fluoridation as a Public Health Measure, *December 28, 29.*

AAAS Subsection Np-Pharmacy:

Six sessions, co-sponsored by the American Association of Colleges of Pharmacy, American College of Apothecaries, American Society of Hospital Pharmacists, and the Scientific Section, American Pharmaceutical Association, *December 27–29.*

AAAS Section O-Agriculture:

Four-session symposium, Public Health Aspects of Soil Science, *December 27, 28.*

PROGRAMS OF SOCIETIES

Oak Ridge Institute of Nuclear Studies:

Two-session symposium on Radioisotopes in Medicine, luncheon, *December 28;* a series of lectures either *December 27 or 29.*

American Society of Protozoologists:

Eight sessions of papers, a luncheon, and a business meeting, *December 27–29.*

American Society of Zoologists:

Fourteen sessions of papers, *December 28, 29,* also symposium, *December 27;* demonstrations, *December 28;* zoologists' dinner and biologists' smoker, evening of *December 29;* and symposia, *December 30.*

Herpetologists' League:

Conference session, *December 28.*

Society of Systematic Zoology:

Five sessions including breakfast, business meeting, and symposium, The Classification of Animals, *December 28;* two paper-reading sessions, *December 29.*

American Microscopical Society:

Three sessions, *December 27–29,* including a symposium on Microscopy.

Biometric Society, Eastern North American Region:

Six sessions of papers on Biometry, *December 27–29.*

Society for Research in Child Development and AAAS Section N:

Four sessions including a symposium, Biochemistry of Nutrition in Human Growth, *December 27.*

Alpha Epsilon Delta Premedical Honor Society:

Luncheon and afternoon program, *December 28.*

American Industrial Hygiene Association:

A special program in connection with inauguration of the new AAAS Section P-Industrial Science.

SPECIAL SESSIONS

AAAS Presidential Address:

By Roger Adams, *December 28.*
Evening lectures of Sigma Xi, and RESA, *December 26, 27.*
Evening lectures of Phi Beta Kappa, and Phi Kappa Phi, *December 29, 30.*

AAAS Annual Exposition of Science and Industry:

The 1951 edition of the Association's Exposition again will total about 150 booths and will fill the arena of Philadelphia's Convention Hall. In addition to leading publishers, instrument makers, and suppliers of scientific materials, there will be technical exhibits by prominent manufacturers of pharmaceuticals and biologicals. On or near the exhibit area will be the Fifth Annual International Photography-in-Science Salon and

the AAAS Science Theatre which, almost continuously, will show the latest foreign and domestic scientific films.

Housing:

Coupons for hotel reservations will appear in *Science*, beginning with the issue of August 24, and *The Scientific Monthly*, beginning September.

Registration:

Main Registration at Convention Hall; fees, $2.00 for members of the AAAS, spouses, and bona fide students; $3.00 for nonmembers. Advance registrants pay 25¢ more but receive the General Program early in December, by first-class mail.